Dear Reviewers,

We initially want to thank you for your valuable time that you spend in order to perform the current peer-review. Below you will find our corrections to all your suggestions. Your suggestions were very valuable and we think that with the performed corrections the quality of the manuscript has been significantly improved.

Our corrections have been high-lighted with yellow in the submitted revised manuscript. Below you can check our performed actions in our manuscript.

Reviewer #1: The manuscript Mallis P et al. entitled "Advances in Mesenchymal Stromal Cell delivery as a potential therapeutic strategy against COVID-19: Preliminary evidence from in vitro results" is well-written, with proper methods and interesting results.

1. **Altough the authors highlighted that this is a preliminaty finding, and the potential for the anti-inflammatory effects, and prevention the overactivation of the immune responses by Mesenchymal Stromal Cell in COVID-19, the authors should explore more the possible implications of this findings to the treatments of SARS-CoV-2-infected individuals.**

Author's Response

Dear Reviewer, we appreciate your valuable comments to our submitted manuscript. As you noticed this study includes our initial preliminary results regarding the potential use of MSCs in critically-ill COVID-19 patients. Until now, there exists a number of clinical trials that have utilized the current proposed advanced cellular therapy in COVID-19 patients. However, the underlying molecular mechanisms, that the MSCs are using for the mediated immunomodulation still need further evaluation and clarification. Currently, a great number of review articles are existing, but basic research articles deciphering the immunomodulatory properties of MSCs are limited. Considering all these facts, me and the rest of the authors wanted to provide further knowledge regarding all these underlying mechanisms of MSCs, thus may be considered as a possible alternative treatment option for COVID-19 patients. Regrading the implications and adverse events that may be followed by administrated MSCs, are considered to be mild to moderate. MSCs are considered as advanced therapeutic medicinal products (ATMPs) hence clinical trials must be established in first, before their potential application. The majority of the clinical trials have demonstrated that either autologous or allogeneic MSCs are safe and well tolerated by the COVID-19 patients. In the majority of the performed clinical trials the adverse events include mostly the increased risk of transient fever. However, more sever adverse events such as acute infusional toxicity, infection, pulmonary embolism, death and tumor development have not been reported after the MSCs infusion (allogeneic or autologous).

The following corrections have been performed to the manuscript.

Section: Discussion, page 24: MSCs currently are considered as Advanced Therapeutic Medicinal Products (ATMPs), hence clinical trials establishing the safe and tolerability of these cells must be conducted. In the majority of the clinical trials, allogeneic or autologous MSCs are intravenously (IV) administrated in COVID-19 patients. Possible adverse events (AEs), that are associated with the IV administration include fever risk, toxicity, infection, pulmonary embolism and possible malignancy formation. However, in the currently conducted clinical trials utilizing the MSCs as possible COVID-19 treatment strategy, only increased risk of fever has been reported. Furthermore, after MSCs IV administration, the AEs are considered as mild to moderate. Besides the aforementioned AEs, other incidences also have been reported and evaluated for the possible relation with MSCs administration. In the study of Shi et al., reported an increase in lactic acid dehydrogenase, serum alanine aminotransferase, creatine phosphatase, aspartate aminotransferase, uric acid and reported hypokalemia, during the 1-year follow-up. However, all these AEs were on site judged and considered as unrelated to the MSCs administration. Considering these data, MSCs are safe and tolerable therapy, therefore more

clinical trials (phase I, II) have been registered (www.clinicaltrials.gov) and currently performed, to further evaluate the potential application of these cells in critically-ill COVID-19 patients.

Section: Conclusion, page 25: Allogeneic MSCs are considered immune-evasive cells, as they are not expressing either the HLA-DR or stimulatory (CD40) and co-stimulatory molecules (CD80 and CD86) [22, 26]. Therefore, their infusion in human subjects should be considered safe. Furthermore, Avanzini et al.[83] showed that MSCs are negative for the ACE2 and TMPRSS2, thus can evade the intrabody COVID-19 infection. This may represent an additional benefit for the application of MSC therapy in critically-ill COVID-19 patients, reversing in this way the manifestation of the current disease.

2. The authors should disccuss more the importance of this treatment, delivery routes of treatment, or the treatment should be performed in vitro and cells transferred to the sick person, etc.

Authors Response

The comment of the reviewer is very important. We think that extensive discussion regarding the importance of the MSC therapy, delivery routes and if initial in vitro priming of MSCs have been extensively discussed throughout the whole manuscript. Also, in conclusion section all this information are included. We have additionally added the following sentences in the discussion section, thinking that the reviewer's comments have been successfully answered.

The following corrections were performed in the manuscript.

Section: Discussion, page 25: "Besides the immunomodulatory properties, MSCs possess key differentiation capabilities, committed mostly to mesodermal lineage cell types. In this way, MSCs can act both in immune regulation of the overactivated immune responses and in alveolar epithelium regeneration. The latter may be related with rapid reverse of lungs' ground glass opacity, which consists major underlying disorder in critically-ill COVID-19 patients. Additionally, in this study it was shown that MSC therapy can be quickly administrated to COVID-19 patients, upon demanding. Therefore, allogeneic MSCs can be isolated, expanded at great numbers and cryopreserved over long time. Upon IV administration to patients, MSCs can be activated by the microenvironment stimuli, therefore no need for initial in vitro priming is required. MSCs should be considered as a safe alternative therapeutic option, which may improve the COVID-19 patients' condition and result to less loss of life."

Section: Conclusion, page 25: "MSCs may also be utilized efficiently in the recovery phase of COVID-19 patients. COVID-19 patients are suffering from extensive lung fibrosis and multiorgan damage of variable severity. Importantly, MSCs after their IV infusion, a) initially widespread in the body through the systemic circulation, b) accumulated early in the lungs and then in spleen and liver, c) migrate to the injury or inflamed sites d) and finally persist to the migrated tissue for a short time before their clearance [32]. In such a way, and due to accumulation to the lung capillary network, MSCs can give rise to differentiated cells such as endothelial and epithelial cells, which can replace the damaged tissue [32]. The latter may be translated to less required recovery time for the COVID-19 patients."

Reviewer #2

1. **-The title seems, for me, a little confusing. The contrast between Advances … and … Preliminary creates a strange combination, although there are many articles on this topic that can be found by searching on literature databases. The subject is exciting and comprises valuable data regarding COVID-19**

Author Response

Initially, we would like to thank the reviewer for comprehensive review to our submitted manuscript. We find the comments very interesting and we tried with our responses to satisfy the reviewer's queries. Regarding the manuscript title we make the following change.

Advances in Mesenchymal Stromal Cell delivery as a potential therapeutic strategy against COVID-19: Promising evidence from in vitro results.

We change the term preliminary with the term promising. In this way, we think that no contrast between the term Advances and Promising evidence may exist. Furthermore, the title must be novel enough in order to avoid plagiarism and to be accepted by the journal.

2. **Accurate data provided for a specific time are irrelevant in the article's subject development, giving the pandemic evolution and many other factors. Few general remarks are sufficient, and more detailed data can be obtained from dedicated platforms.**

Author's Response

According to reviewer's comment the following sentence was removed.

Section: Introduction, page 4: "The United States of America (USA) exhibited the highest number of new COVID-19 cases per 1 million people (121,016), and a total number of deaths (659,924), worldwide, followed by India, Brazil, Russia, the United Kingdome and others [6, 7]."

The following sentence was added in the same section, assuming that we are fulfilling the reviewer's suggestion.

Section: Introduction, page 4 : "Now, COVID-19 represents a major global issue, counting more than 281,484,620 total cases and more than 5,409,113 fatalities, since the initial outbreak [6]. Indeed, the COVID-19 pandemic has spread in more than 220 countries. [6, 7]. Accurate date regarding the worldwide spread of COVID-19 can be provided global monitoring platforms such as Johns Hopkins University Coronavirus Research Center [6]"

3. **In the abstract is stated that morbidity is less than 6%. However, in the above paragraph, mortality is indicated at the same level. Therefore, it is necessary to be clarified this aspect.**

Author's Response

We totally agree with the reviewer's comments. The right term to be used in both abstract and introduction sections is the mortality rate and not morbidity. Mortality rate is referred to the percentage of the deaths due to a specific disorder. There exist also other statistics terms such as the case fatality rate (CFR), but we assume that all these statistic data are far beyond of the context of the submitted manuscript.

The following changes were performed in the main manuscript.

Section: Abstract, page 2: "COVID-19 is characterized by a low mortality rate (< 6%), however, this percentage is higher in elderly people and patients with underlying disorders."

Section: introduction, page 5: ”COVID-19 is currently characterized by an average mortality rate of less than 6% globally, however, in ages above 65 years old or patients with significant underlying disorders, the mortality rate is increasing dramatically [6, 10,11].”

Section: Introduction, page 6: “Considering the great prevalence of COVID-19, more therapeutic strategies, targeting the aberrant host immune responses, must be evaluated.”

The expression “and its great morbidity and mortality rates globally” has been totally removed from the above sentence.

In the whole manuscript only the term mortality is existed currently.

4. **The explanatory link between the characterization of MSCs and their use as a cell therapy for COVID-19 need a more detailed argumentation given the intense attention they receive from many researchers in their articles, as stated above: over 80 clinical trials for COVID-19 and as also sustained by the authors: "interplay between MSCs and hyper-stimulated immune cells in COVID-19 patients has not been satisfactorily explained."**

Author’s Response

We totally agree with the reviewer’s suggestion. We think that the following paragraphs are providing enough evidence regarding the link between the characterization of MSCs and their potential use as cell therapy in critically-ill COVID-19 patients.

Section: Introduction, pages 6-8: “MSCs are non-hematopoietic stem cells with great immunoregulatory/immunosuppressive abilities. MSCs represent a mesodermal multipotent stem cell population, which initially was discovered in Bone Marrow (BM) aspirate samples by Friedenstein et al. [21]. Currently, MSCs can be obtained from various sources of the human body, including liver, lungs, adipose tissue (AT), umbilical cord blood, placenta, and umbilical cord tissue (Wharton’s Jelly-WJ tissue) [22]. Based on the proposed guidelines of the MSC Committee of the International Society for Cell and Gene Therapy (ISCT), MSCs must fulfill specific criteria [23, 24]. Briefly, MSCs must exhibit a) plastic-adhesion ability (spindle-shaped cells), b) tri-lineage differentiation towards “osteocytes”, “chondrocytes” and “adipocytes” under defined conditions and c) specific immunophenotype [23, 24]. Interestingly, MSCs are characterized by positive and negative expression of specific cell surface markers (clusters of differentiation- CDs). MSCs express > 95% of CD73 (5’-nucleotidase), CD90 (Thy-1 antigen), CD105 (endoglin) and < 2% of CD34 (hemopoietic stem cell marker), CD45 (pan-lymphocyte antigen), HLA-DR (HLA class II molecules), CD11b (macrophage marker) and CD19 (B-lymphocyte marker) [23, 24].

Also, MSCs are considered as immune-evasive stem cells, thus cannot be recognized by the immune cells e.g. macrophages, T and B cells [25, 26]. Intriguingly, the immune evasion of MSCs is elicited mainly by the lack of HLA class II molecules and costimulatory molecules such as CD80, CD86, CD40, and CD40 ligand [25, 26]. Besides, the proposed guidelines by ISCT, MSCs from different sources are characterized by variable functional properties. Indeed, fetal MSCs (e.g. derived from amniotic fluid-AF, placenta-P, and WJ tissue) may have significant differences in terms of proliferation and differentiation efficiency, telomere length, and telomerase activity, compared to adult MSCs (e.g. adipose tissue and bone marrow) [22, 27-29]. Also, it has been shown that fetal MSCs are characterized by better immunoregulatory/ immunosuppressive properties and have acquired less mutagenic or epigenetic changes to their genome, in comparison to MSCs derived from adult sources [22, 27-29]. MSCs are known for their immunoregulatory properties, mediated either through the cell-cell contact mechanisms and through the secretion of bioactive molecules [30]. MSCs have broad effects on the cells of innate and adaptive immunity. Specifically, MSCs can orchestrate the phenotype switching from proinflammatory M1 to anti-inflammatory M2 macrophages, promote the production of tolerogenic DCs, T and B cell inhibition [31]. These functions can be mediated either through direct contact of MSCs with the immune cells and activation of cell signaling pathways (promoted after cell contact interactions) such as Fas/ Fas ligand, TNF-α/ TNF-R, PD-L1/PD-1, and HLA-G) or through the release of specific molecules e.g. indoleamine-2.3-dioxygenase (IDO), nitric oxide (NO), galectins and the soluble forms of HLA-G (HLA-G5-G7) [31]. Currently, MSCs have been utilized in over 80 clinical trials for COVID-19, registered to the international database clinicaltrials.gov (www. clinicaltrials.gov) [32, 33]. In the majority of the studies, the safety and efficiency of the infused MSCs have been well evaluated [32-37]. However, in those studies, the exact interplay between MSCs and hyper-stimulated

immune cells in COVID-19 patients has not been satisfactorily explained.

Furthermore, due to the mesodermal lineage differentiation capacity of MSCs, these cells may exert beneficial tissue regeneration of the damaged tissue."

Section: Discussion, pages 24-25: "MSCs currently are considered as Advanced Therapeutic Medicinal Products (ATMPs), hence clinical trials establishing the safe and tolerability of these cells must be conducted. In the majority of the clinical trials, allogeneic or autologous MSCs are intravenously (IV) administrated in COVID-19 patients. Possible adverse events (AEs), that are associated with the IV administration include fever risk, toxicity, infection, pulmonary embolism and possible malignancy formation. However, in the currently conducted clinical trials utilizing the MSCs as possible COVID-19 treatment strategy, only increased risk of fever has been reported. Furthermore, after MSCs IV administration, the AEs are considered as mild to moderate. Besides the aforementioned AEs, other incidences also have been reported and evaluated for the possible relation with MSCs administration. In the study of Shi et al., reported an increase in lactic acid dehydrogenase, serum alanine aminotransferase, creatine phosphatase, aspartate aminotransferase, uric acid and reported hypokalemia, during the 1-year follow-up. However, all these AEs were on site judged and considered as unrelated to the MSCs administration. Considering these data, MSCs are safe and tolerable therapy, therefore more clinical trials (phases I, II and III) have been registered (www.clinicaltrials.gov) and currently performed, to further evaluate the potential application of these cells in critically-ill COVID-19 patients."

Section: Conclusion, pages 25-26: "In conclusion, MSCs derived either from WJ or BM, can exert key immunoregulatory functions towards inflammation. SARS-CoV-2 have a broad effect in patients' body, orchestrating the production of the pro-inflammatory cytokines and, also inducing extensive damage to alveolar epithelial cells [82]. MSCs are currently used in a great number of clinical trials, ameliorating efficiently the immune system dysregulation [32-37]. Importantly MSCs from BM were characterized by lower production of the studied immunoregulatory agents compared to WJ-MSCs. However, more research is required, to characterize better the immunoregulation mediated by MSCs from various tissue sources. WJ-MSCs possess more naïve cells compared to MSCs derived from adult sources. Moreover, it has been shown that MSCs derived from fetal tissues are characterized by fewer mutations and epigenetic modifications, greater proliferation, and differentiation capacity, compared to the adult MSCs [22] In addition, MSCs from fetal tissues can be isolated noninvasively (compared to adult MSCs) [22]. Allogeneic MSCs are considered immune-evasive cells, as they are not expressing either the HLA-DR or stimulatory (CD40) and co-stimulatory molecules (CD80 and CD86) [22, 26]. Therefore, their infusion in human subjects should be considered safe. Furthermore, Avanzini et al.[83] showed that MSCs are negative for the ACE2 and TMPRSS2, thus can evade the intrabody COVID-19 infection. This may represent an additional benefit for the application of MSC therapy in critically-ill COVID-19 patients, reversing in this way the manifestation of the current disease. MSCs may also be utilized efficiently in the recovery phase of COVID-19 patients. COVID-19 patients are suffering from extensive lung fibrosis and multiorgan damage of variable severity. Importantly, MSCs after their IV infusion, a) initially widespread in the body through the systemic circulation, b) accumulated early in the lungs and then in spleen and liver, c) migrate to the injury or inflamed sites d) and finally persist to the migrated tissue for a short time before their clearance [32]. In such a way, and due to accumulation to the lung capillary network, MSCs can give rise to differentiated cells such as endothelial and epithelial cells, which can replace the damaged tissue [32]. The latter may be translated to less required recovery time for the COVID-19 patients. "

Also, the following paragraph was added to the Introduction section.

Section: Introduction, page 8: " Furthermore, due to the mesodermal lineage differentiation capacity of MSCs, these cells may exert beneficial tissue regeneration of the damaged tissue. The pathogenesis of COVID-19, involves the injury of the alveolar epithelium, which further may induce lung fibrosis, a state which is known as ground glass opacity. MSCs can either be differentiated to endothelial and epithelial cells or can direct the differentiation of epithelial and endothelial progenitor cells, through a paracrine manner. MSCs, can exert both immunoregulatory properties and tissue regeneration abilities, therefore their use as alternative therapeutic strategy in critically-ill COVID-19 patients, must be strongly considered by the physicians."

We assume that with the above paragraphs and also with the latest addition to provide specific details regrading the link between MSCs characterization and their potential utilization in COVID-19. We did

not want to add more details, because this manuscript represents an original research work and not a review or a meta-analysis study.

5. **" Cytokine storm" is a dominant paradigm in explaining the pathogenesis of SARS-CoV-2 infection. However, it remains unclear to me if MSCs can calm this turbulent immunological imbalance that can deteriorate the internal homeostasis of the patient's body. On this fundament, haw are acting these cells, what they are doing and what are their functional limits in reducing or calming the earlier mentioned " Cytokine storm"?**

Author's Response

We thank the reviewer for this question. Cytokine storm in COVID-19 patients is initiated due to the aberrant immune responses cause by the SARS-COV-2 pathogenesis. In critically-ill patients overactivating immune responses which results to increase production of IL-2, IL-6, IL-7, G-CSF, IP10, MCP1, MIP1A, and TNF-α, have been observed. In this context, and as we showed in the introduction section MSCs are characterized by specific immunoregulatory/ immunosuppressive properties which can be exerted upon specific stimulation of these cells. In this way, when the MSCs are located in an inflammatory microenvironment, such as those that can be caused by SARS-COV-2, then these cells are stimulated and exert key function immunoregulatory actions, either through direct or indirect contact. This actually was also the main of this study, to evaluate the immunoregulatory properties of WJ and BM-MSCs upon stimulation with culture medium containing COVID-19 serum. So, the results of our study clearly indicated the increased secretion levels of IL-1RA, IL-6, IL-10, IL-13, TGF-β1, FGF, PDGF, VEGF-A and IDO by both MSCs' sources. Due to our result, we believe, that a specific interplay of MSCs and overactivated immune response is performed, regulating successfully the over-stimulated immune cells of COVID-19 patients. The specific interplay between MSCs and activated immune responses has been in detail explained in the whole discussion section. Also, we have supporting evidence from currently performed clinical trials, regarding the beneficial actions of MSCs in critically-ill COVID-19 patients. Please check the revised version of the manuscript and more specifically the discussion section, in order to verify the these details. So, until now a great number of clinical trials have been performed, showing the beneficial properties of MSCs in halting the induced cytokine storm in critically-ill-COVID-19 patients. However, in all these clinical trials, there was lacking evidence, regarding the exact underlying molecular mechanism of how MSCs are working and implicating in all these process. Until date, still there are only a few studies that have evaluate the immunoregulatory actions of MSCs, and more importantly, this may be among the first studies which tries to explain the missing link regarding the immunoregulatory properties of MSCs and beneficial effects in COVID-19 patients. We think, that no additional information, by our side is required, and we assume that with our revised version of the manuscript and the discussion, we offer explanatory information regarding the underlying molecular mechanism of MSCs in tolerating the aberrant immune responses in COVID-19 patients. Furthermore, we didn't want to expand more the discussion section, because this study represents an original research article and not a review. Please read carefully our submitted revised manuscript version, both introduction and discussion sections.

6. **It was great to read, understand, and evaluate this high-level, scientifically challenging article with a high-performance experimental design.**

Author's Response

In the end, we want gratefully thank the reviewer for the very kind words that shared with us regarding our work. This work is very valuable for us, and deserves to be published. If any other corrections need to be performed, we are more than willing to perform them in order to achieve the publication goal.

Reviewer #3

Mallis P et al present in their current manuscript the mesenchymal stromal cell delivery as a potential therapeutic strategy against COVID-19. The presented study lacks the novelty, pioneering and in-depth molecular investigation levels. At the same time, I think the author lacks the rigor and objectivity of scientific researchers. This article is not for consideration of publication in the World Journal of Stem Cells.

Author's Response

Personally me and the rest of the authors, were very disappointed regarding the reviewer's opinion. The previous 2 reviewers shared with us so kind words about our research study. Both reviewer 1 and 2 found the study novel enough with high significant background. I am sorry that even the 3rd reviewer commented specific suggestions in order to have the chance to make the appropriate corrections. Even now, we are more than willing to perform further changes if the third reviewer wants.

We have checked the whole manuscript for any grammar or language expression error and we corrected if it was required. Furthermore, we updated the reference order.

We think with the above performed corrections, that the quality of the manuscript has been improved and can be processed to the next step of the publication process.

We are more than willing to perform more corrections, in order the manuscript to get published.

Again, we would like to thank the reviewers for the performing peer-review.

Yours sincerely,

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