Palermo, March 07, 2023

Dear Editor,

we greatly appreciated your timely, rigorous, and thoughtful reviews of our manuscript entitled "Different Priming Strategies Improve Distinct Therapeutic Capabilities of Mesenchymal Stromal/Stem Cells: Potential Implications for Their Clinical Use" (Journal: *World Journal of Stem Cells*; NO: 83491). We have revised the manuscript, according to the comments and suggestions of reviewers, and answered the questions in a point-by-point fashion as listed below. The major changes were highlighted in the revised manuscript. We hope our responses have adequately addressed the concerns.

Once again, we express our appreciation for your work on our manuscript.

Sincerely,

Vitale Miceli
Research Department
IRCCS-ISMETT (Mediterranean Institute for Transplantation and Advanced Specialized Therapies)
Via Tricomi 5, 90127 Palermo, Italy
Phone number: +39 091 2192496
email: vmiceli@ismett.edu

Author's Response to Reviewers' Comments:

Reviewers 1

The authors reviewed articles to address the impact of different priming treatments, including inflammatory cytokines, hypoxia, and three-dimensional physical cues on the MSCs from different origins and to enhance the therapeutic potential of the MSCs. In addition, the articles included comprehensive tables and figures to summarize and compare the impact differences.

1) The authors mentioned the impact of MSC heterogeneity on the poor efficacy in clinical trials. However, the authors didn't address the heterogeneity clearly, which was from the tissue differences or within the same tissue. From the recent single-cell articles, we gained insight into the detailed architecture of stromal heterogeneity from different tissues. An example would be Buechler et al. Nature 2021, https://www.nature.com/articles/s41586-021-03549-5.Authors may consider further addressing the impact of priming treatment on which specific subpopulations and differences. Some suggested articles include Kosaric et al., Molecular Therapy, 2020 https://www.cell.com/molecular-therapy-family/molecular-therapy/fulltext/S1525-0016(20)30286-0; Cai et al. Cell & Bioscience, 2022 https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-022-00848-w.

   Response: We thank the reviewer for carefully reading the manuscript and for this useful suggestion. As highlighted on page 5 lines 27-29 (introduction), and page 19 lines 20-21 and 25-27 (conclusions), we discussed about MSC heterogeneity, emphasizing that it is related to both different MSC origin and the use of diverse harvesting and culture strategies. We also discussed the priming strategies as a useful tool for eliminating heterogeneity of MSCs. Appropriate references were also added.

2) The authors organized a comprehensive table summarizing the priming treatment effect on the MSCs. However, the order of the cytokine section was

neither by MSC types nor priming treatments. I would suggest authors categorize the same MSC type or priming treatment together.

Response: We thank the reviewer for the comment. We organized table 1 by both priming treatments and reference number.

3) The author might consider using the same citation format in the table. Consider replacing the ['last name'; 'year'] format with the number used in the article, such as Bulati et al. 2020 with "Ref 38" and Garcia et al. 2019 with "Ref 127". Since the references were not ordered by last name, doing so may help readers locate the original article much more quickly.

Response: We thank the reviewer for the suggestion. We changed the current citation format with the reference number.

Reviewers 2

This manuscript focused on the topic "Different priming strategies improve distinct therapeutic capabilities of mesenchymal stromal/stem cells" and reviewed the therapeutic properties of primed MSCs in preclinical models. However, there are some issues that need to be addressed.

Firstly, the author published a review titled " Therapeutic Properties of Mesenchymal Stromal/Stem Cells: The Need of Cell Priming for Cell-Free Therapies in Regenerative Medicine" in 2021, which had described three methods for MSCs priming. So, it's hardly to find novelty in this manuscript.

Response: We thank the reviewer for carefully reading the manuscript and for putting out this question.

In the 2021 review, we discussed both the need of cell priming to enhance MSC properties and the opportunity to use secretome components as a tool for the implementation of cell-free therapies. In that review, we analyzed the literature from a molecular point

of view, showing that several priming strategies are useful for enhancing the production of specific functional factors, which in turn lead to potentiate biological functions of MSCs. In the 2021 review, we did not point out that one specific priming method is different from another regarding the use of MSC therapy in a specific category of disease. The "take home message" of the past review was: "There is potential for improvement in MSC treatment and pretreating cells prior to use as therapeutic tool appear to be a promising strategy".

In our new review, we resume the basic concepts of the previous manuscript, but we analyzed the literature describing how different types of priming can be differently effective in specific preclinical models of disease. We wanted to emphasize that specific priming strategies can make MSC therapy more appropriate for specific categories of diseases. The "take home message" of this new review is: Different priming strategies can be used to direct the therapeutic effects of naïve MSCs toward specific disease models (acute or chronic).

While many paper have discussed the potential application of MSC-based therapy on different pathological conditions, in our review, we highlighted that, to get the best MSC efficacy, appropriate priming strategies are needed to treat specific diseases.

1) The abstract is lengthy, with 255 words, which is not concise enough.

Response: We thank the reviewer for the comment. We reduced abstract words from 255 to 200.

2) In the second paragraph of the introduction, the clinical products of MSCs should focus more on the controversy section of "MSCs have moderate or poor efficacy, and the results from some studies are controversial".

Response: We thank the reviewer for carefully reading the manuscript and for this useful suggestion. As highlighted on page 5 lines 27-29 (introduction), and page 19 lines 20-21 and 25-27 (conclusions), we discussed about MSC heterogeneity, emphasizing that it is related to both different MSC origin and the use of diverse harvesting and culture strategies. We also discussed the priming strategies as a useful tool for eliminating heterogeneity of MSCs. Appropriate references were also added.

3) In the section "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs": 1) extracellular vesicles are not soluble factors; 2) The narrative order is a bit out of order, the original text is exosomes - soluble factors - exosomes, it is recommended to adjust.

Response: We thank the reviewer for the comments. We removed "soluble factors" referring to extracellular vesicles and changed the narrative order of the section "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs"

4) In the section "THERAPEUTIC PROPERTIES OF PRIMED MSCs IN PRECLINICAL MODELS", there is a commonsense error: sepsis is not a chronic disease.

Response: We thank the reviewer for carefully reading the manuscript. We removed sepsis from chronic disease list.

Reviewers 3

The current review is focused on the specific priming strategies that have been implemented to improve the regenerative and immunomodulatory properties of MSCs. The production of priming type-specific functional factors in MSCs could improve the effectiveness of MSCs in clinics and pave the way toward implementing new MSC-based therapies. Overall, the manuscript is well written and interesting, but still some improvements are required.

Response: We appreciate the reviewer interest in our work and we will take care of the critiques and suggestions raised.

1) "Mesenchymal stromal/stem cells (MSCs) have shown significant therapeutic potential and have therefore been extensively investigated for application in the field of regenerative medicine." is contradictory with "Unfortunately, while MSCs have shown a good margin of safety as a cellular treatment, they have usually been therapeutically ineffective in human diseases." mentioned in Page 2 Line 2-9.

Response: We apologize to the reviewer for being unclear. We modified those sentences (Page 3 lines 3-5). In this part of the paragraph, we would like to emphasize that, despite the therapeutic potential of MSCs are largely tested on animal preclinical studies showing promising results, the same results have not been fully confirmed on human studies.

2) "extracellular vesicles (EVs)" mentioned in Page 5 Line 3 should be changed to "EVs".

Response: We thank the reviewer for the comment. We changed "extracellular vesicles (EVs)" with "EVs"

3) The statement in the chapter of "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs" should be adjusted appropriately. Otherwise, it looks like each sentence has nothing to do with the next sentence.

Response: We thank the reviewer for carefully reading the manuscript. As suggested by the reviewer, we modified the paragraph "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs"

4) The format of the Table 1 should be standardized, and it is recommended to classify the contents of Table 1 for easy reading.

Response: We thank the reviewer for the suggestion. We modified table 1 according to reviewer's suggestions.

5) In Page 14 Line 4-38, besides stating your views according to the published literature, please also mention the inference of aggregating sentences to avoid the sentences having nothing to do with the next sentence.

Response: We thank the reviewer for the suggestion. We modified this paragraph according to reviewer's suggestion.

Reviewers 4

In this review, the authors reviewed the effects of MSC on chemoattraction and modulation of inflammation, angiogenesis, and tissue repair under three priming strategies: inflammatory cytokines, 3D cultures, and hypoxia. The literature is comprehensive but there are several important issues that need clarification.

Response: We appreciate the reviewer interest in our work and we will take care of the critiques and suggestions raised.

1) Choosing more specific nouns that summarize article information as keywords would be better.

Response: We thank the reviewer for the suggestion and we changed the keywords

2) The background is disorganized. After introducing the efficacy of MSC, the clinical trial results can be summarized to explain the current bottlenecks of MSC treatment, such as heterogeneity, low migration to injured tissues, and then to introduce the priming strategies.

Response: We thank the reviewer for the suggestion and we changed the background according to reviewer's suggestion

3) The dosages form of MSC were suggested to added in the table 1, cells or

exosomes.

Response: We thank the reviewer for the useful suggestion. We added the dosage form in table 1

4) It will goes deeper if a Figure 3 that shows different priming strategies through different signaling pathways regulate MSC is prepared.

Response: We thank the reviewer for the useful comment. We added a new figure 1 illustrating how different priming strategies through different signaling pathways regulate the MSC phenotype.

Reviewers 5

Mesenchymal stromal/stem cells (MSCs) have demonstrated promising therapeutic results in the field of regenerative medicine. In this study, the authors reviewed data on the principal priming approaches for enhancing the therapeutic potential of MSCs. The study is logically designed, the idea is new and very interesting. Although, there are several concerns that need to be addressed.

Response: We appreciate the reviewer interest in our work and we will take care of the critiques and suggestions raised.

1) I think more work is needed in the section of "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs". In the section of "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs", it is better to go into more detail on the introduction of exosomes, some latest references could be cited, "Exosomes as mediators of intercellular crosstalk in metabolism", "Exosomes Regulate the Epithelial-Mesenchymal Transition in Cancer", for

example, or any other similar references.

Response: We thank the reviewer for the useful comment. We modified the section "THE SECRETION OF PARACRINE FACTORS MEDIATE THE THERAPEUTIC FUNCTION OF MSCs". We also introduce EXOs function as key components of intercellular communication, as they are released into the intercellular space where they exert local paracrine or distal systemic effects (page 7 lines 7-9).

2) In the "Priming with 3D culture of MSCs" section, is it "omic approaches" or "omics approaches"?

Response: We thank the reviewer for the comment. We changed "omic approaches" with "omics approaches"