

## EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

### *(1) Science editor:*

This manuscript by Dr. Maurya and colleagues investigated the potential radioprotective effects of hWJ-MSCs and CM by identifying selected soluble factors responsible for the observed effects. The study highlighted the value of G-CSF in mediating therapeutic properties. This work adds new knowledge that can advance the potential use of MSCs as a cell therapy to prevent acute radiation syndromes. The title is informative and the topic is interesting and well presented. The methods are well reported and conclusions are consistent with results. The study is well written and worth publishing after minor revision.

**Response:** Authors would like to thank all the reviewers and the editor for critical going through the manuscript and providing us with constructive comments.

### Reviewer #1

Materials and methods: Please refer related previous study on the methods that you use. Please provide your reasoning for determining the radiation dose of 6Gy and 8.5Gy.

**Response:** A dose of 6 Gy was used in our study as sub-lethal dose that can induce lymphopenia in mice. Inducing lymphopenia in the host allow graft lymphocytes to divide via homeostatic driven proliferation (HDP). This dose of 6 Gy was also used in spleen CFU experiment which is a gold standard assay to demonstrate radioprotection. In this assay, mice are exposed to sub-lethal dose so that colony forming ability of hematopoietic stem cells can be seen as macro-colonies on the spleen. Lethal dose of 8.5 Gy was employed while investigating the therapeutic radioprotective efficacy of conditioned media (CM) wherein the survival of mice was used as an end point.

Please provide your reasoning for using 200 microliters of conditioned medium in the survival study. If the doses are found from your previous study, please clarify.

**Response:** Selection of 200ul conditioned medium was based on our previously published report. We have used both 100 and 200 µl of conditioned medium, however, optimum outcome was observed when 200 µl was used (Bandekar et al., Am J Transplantation, 2020).

How many mice were involved in your study? Please provide details about the animals used.

**Response.** Information about the number of mice used is mentioned in the legends to Figure 2 and 3. For mice survival study, each group comprises of 12 mice whereas for endogenous colony forming assay, each group comprises of 5 mice. A total of 4 mice in each group was used in homeostasis driven proliferation experiment.

About the infusion procedure, please provide details about the procedure and duration for better understanding.

**Response:** WJ-MSC-CM or WJ-MSC-CM neutralized with anti-G-CSF were systemically infused through the lateral tail vein 24h post radiation exposure in both survival and endogenous spleen colony experiments. The same is described in the respective experimental methods in the manuscript. We have now incorporated detailed infusion procedure for homeostasis driven proliferation experiment in the revised manuscript.

Please specify the statistical methods used.

**Response:** We have added information about statistics used under Methods section in the revised manuscript.

Discussion: Please provide the limitations of your study within the methodology.

**Response:** We thank the reviewer for the suggestion. The limitations of our study are as follows: 1. Administration of WJ-MSC-CM to lethally irradiated mice offered only about 40% protection indicating that other mechanisms are also playing a role in WJ-MSCs mediated radioprotection. Alternatively, the concentration of G-CSF present in the WJ-MSC-CM may not be equivalent to G-CSF secreted by infused WJ-MSCs in vivo. This limitation is now incorporated in the revised manuscript.

2. The present study demonstrates therapeutic radioprotection of conditioned medium collected from culturing WJ-MSCs in mice. However, these findings need to be validated in rats and non-human primates.

References: Please follow the format for references guidelines.

**Response:** The references are formatted as per journal style in the revised manuscript.

Figures: Please ensure clear and sharp figures for better viewing, in particular figure 6, 7, 8, and supplement figures.

**Response:** We have now provided better resolution figures in the revised manuscript.

### **Reviewer #2**

Grammar: Need some revision. (Check the attached file from Reviewer #2).

**Response:** We have incorporated all the grammatical changes suggested by the learned reviewer in the revised manuscript. We thank the reviewer for keenly going through the manuscript. Further, the revised manuscript is thoroughly checked for grammatical errors.

Please provide the following information in the Paper 1. Conflict of Interest 2. Source of Funding

**Response:** As per the reviewer's suggestion, we have included Conflict of interest and Source of funding information before Acknowledgment section in the revised manuscript.

### **Reviewer #3**

1. In the title the authors define mesenchymal cells as "mesenchymal stem cells" but, over the years, scientists have debated the name of these cells, and the term "mesenchymal stem cells" has often been changed to "mesenchymal stromal cells". The authors should change the term "stem" with either "stromal" or at least "stromal/stem" in the title and along the text.

**Response:** As per reviewer's suggestion, we have now used "mesenchymal stromal / stem cells" in the title and also in the text at first use.

2. On page 6, please extend the acronym HSC in “HSC-niche”

**Response:** We have expanded the acronym HSC in HSC-niche in the revised manuscript.

3. On page 7 (material and methods section), in the paragraph “Isolation and characterization of WJ-MSCs”, a briefly description of WJ-MSCs isolation and phenotypic characterization should be added (a new figure 1 showing MSC images and distinctive markers should be added).

**Response:** We had provided complete description of WJ-MSCs isolation and phenotypic characterization in our published manuscript by Bandekar et al., American Journal of Transplantation, 2020. This reference is part of the Methods section of the manuscript.

4. To prepare the conditioned medium, did the authors try various conditioning times (48 and 72 hours) to verify better efficacy?

**Response:** Preparation of the conditioned medium was based on our earlier published paper in which we have optimized the cell number and culture volume by fixing the time interval at 24h. However, we have not performed experiments by collecting the conditioned medium at 48 or 72h time intervals. We thank the reviewer for this suggestion and will investigate as part of our future studies.

5. In Figure 8, to make the individual gene names for clusters 1, 2 and 3 more readable, the resolution should be improved.

**Response:** We have made it readable by including better resolution images for clusters 1, 2, and 3 for Figure 8.

## ***(2) Company editor-in-chief:***

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Stem Cells, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, “Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...” Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is ‘original’, the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022.

**Response:** Authors would like to thank Editor-in-Chief for conditional acceptance of the manuscript. We have provided point-by-point response to the concerns raised by all the three learned reviewers. All the figures are original and as per the suggestion, we have added “Copyright ©The Author(s) 2022” in the ppt.