# Response to the Reviewer's Queries

**Date:** Aug 25, 2022

**Manuscript Title:** Long-term and non-invasive in vivo tracking of DiD-labeled human hepatic progenitors in chronic liver disease models

**Journal:** World Journal of Hepatology

**Manuscript ID:** 78965

On behalf of all the authors, I would like to thank editors and all the reviewers for their constructive comments, which have helped to refine the manuscript. We are pleased to submit the revised manuscript for your kind perusal. The submission includes the following:

1. Response to all the reviewers' comments
2. **Revised version of the manuscript as supplementary file** (all changes are made in track version)
3. Figure as .ppt with editable figures and legends
4. An audio file

We hope our responses to the reviewers' comments are satisfactory and believe that the revised manuscript is suitable for publication in your esteemed journal. Kindly find below the response to the reviewers' comments. We have made relevant changes to the manuscript.

| S.No | Comments | Response to the Comments |
|---|---|---|
| **Reviewer: 1** | | |
| | Thank you very much for the opportunity to review this manuscript. In this manuscript, the authors evaluated DiD labeling of cells and found this staining could be enable long-term and non-invasive tracking of transplanted cells in vivo up to 80 days. DiD might be the promising staining and further evaluation is needed. The topic is interesting and the manuscript is well written. I have few comments as following; | We appreciate reviewer's effort for careful review and consideration of our manuscript. We have also included the amendments suggested by the reviewer in form of minor errors as follows: |
| | (1) The author should discuss the novelty of DiD in liver transplanted cell more in introduction part. | (1) We have included more discussion for the novelty of DiD in liver transplanted cells in the introduction section. |
| | (2) The full name of DiD and SCID should be added in the abstract part. | (2) We have added the full name of DiD and SCID in the abstract part. |
| | (3) The number/values at the figures are too small and it will be better to increase the font size. | (3) As suggested, we have revised all the figures and increased the font size of each figure for better clarity. |

| **Reviewer: 2** | |
|---|---|
| In this study, lipophilic fluorescent dye DiD-labeled fetal hepatic progenitor cells (fHPCs) were transplanted into chronic liver disease (CLD) mice livers. The results showed that DiD labeling of cells enabled long-term and non-invasive tracking of transplanted cells in vivo up to 80 days. This manuscript provides a new method to understand the homing, distribution, and differentiation into the desired cell types contributing to the organ regeneration. However, there're still two issues which should be addressed.<br><br>(1) "DiD labeling" Section: Why did the authors choose 1 X $10^5$ cells (100µL) to inject? Did you try other dosages?<br><br>(2) Why did you choose human fetal liver cells instead of animal cells?<br><br>So, revision should be recommended for this manuscript. | Thank you for your valuable inputs! Please find the revision and possible explanation for each query.<br><br>(1) In earlier studies, we have used 8 X $10^7$ HPCs for txn in patients with End-Stage Decompensated Liver Cirrhosis (**Khan et al_ Cell Transplantation 2010, DOI: 10.3727/096368909X484707**) which was able to provide marked clinical improvement in terms of all clinical and biochemical parameters. Further, there was decrease in mean MELD score ($p < 0.01$) observed in 6 months follow-up in all the patients. In our another recent study 10×$10^6$ EpCAM+ve human hepatic progenitor cells (hHPCs) were used for complete repopulation of decellularized rat liver scaffolds to get the enough functional response in acute liver failure condition (**Vishwakarma et al_MSCE, 2019, https://doi.org/10.1016/j.msec.2019.01.045**). When we calculated the cell number according to the body weight from the first study, we found that 2 X $10^5$ cells and from the second study 6 X $10^6$ cells should be more than enough to provide sufficient structural and functional response in chronic liver failure condition. Hence, in our current study, we selected slightly lower number of cells (1 X $10^6$) in accordance with the comparatively lesser size of the liver. The volume of 100µL was selected to prevent the extensive damage of the liver arteries and retention of infused cells.<br>We didn't try dose-dependent experiment in this study.<br><br>(2) Since more than two decades, our group is working to harness the potential of human fetal liver cells in different liver diseases, and we have all the available set-up and markers to conduct studies using human liver cells. Hence, we selected human fetal liver cells instead of animal cells. Furthermore, we also wanted to evaluate emergence of any xenogenic response against human cells when transplanted in SCID mice. |

Thank you again for consideration of our revised manuscript!

Sincerely,
Dr. Aleem Ahmed Khan
Scientist & Head
Central Laboratory for Stem Cell Research and Translational Medicine,
Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences,
Kanchanbagh, Hyderabad, Telangana, India