Reviewer 03203589：

1.In the Stem cell transplantation, CD34+ stem cells were obtained at a density of (2.81 ± 1.03) ×106/kg, Is the cell density unit in kilograms?

Response: Thanks a lot for the reviewer's comments, and we are very sorry for this "stupid" mistake. It should be (2.81 ± 1.03) $\times 10^6$ cells/ml.

2.In the Impact of stem cell transplantation on the incidence of HCC, 11 cases with development of HCC in the SCT group, what is the number of case with development of HCC among BMSC transplantation group and HSC transplantation group in the SCT group?

Response: Thanks a lot for the reviewer's comments. In this study, there were 4 cases in the BMSC transplantation group and 7 cases in the HSC transplantation group developing HCC.

3.In the Impact of stem cell transplantation on the incidence of HCC, Can you compare the incidence of HCC between BMSC transplantation group with non-SCT group, and the incidence of HCC between in HSC transplantation group with non-SCT group, and whether the incidence of HCC of stem cell transplantation from two different sources is statistically different? And whether you can draw a conclusion that the incidence of HCC was higher in the SCT group than in the non-SCT group?

Response: Thanks a lot for the reviewer's comments. In this study, the incidence of HCC was 33.3% (4/12) in the BMSC transplantation group and 63.6% (7/11) in the HSC transplantation group, respectively, and there was no significant difference in the incidence of HCC detected between the two groups (P = 0.220).Due to the small sample size, the patients with HCC in both the BMSC and HSC groups were merged, and then the incidence of HCC in the SCT group was compared with that in the non-SCT group. We found higher incidence of HCC in the SCT group than in the non-SCT group (χ^2=8.390, P = 0.015). However, further large-scale RCTs to confirm the findings from this study are warranted.

	BMSC group （n=12）	HSC group （n=11）	Non-SCT group （n=69）	χ^2	P
Incidence of HCC （%）	4（33.3）	7（63.6）	15（21.7）	8.390	0.015

Reviewer 01213078:

The authors reported the long-term efficacy and safety of SCT for decompensated liver cirrhosis by retrospectively analyzing a cohort of patients. The results demonstrated that SCT fails to increase the long-term survival rate and increase the incidence of HCC in patients with decompensated liver cirrhosis. The results of this study is very important because it alert the medical field for the tumorigencity of stem cells in specific condition as liver cirrhosis. Although it is not a RCT study, by it is from real world data and analyzed with proper biostatistics.

Response: Thanks a lot for the reviewer's comments. Indeed, stem cell therapy has shown a short-term efficacy and safety for the treatment of decompensated liver cirrhosis; however, the findings from our retrospective study demonstrate that SCT fails to increase the long-term survival rate and increase the incidence of HCC in patients with decompensated liver cirrhosis. Our data may provide new insights into the choice of SCT for decompensated liver cirrhosis, and we strongly recommend closely monitoring of HCC in those patients undergoing autologous SCT. We also call for multi-center RCTs to further validate our findings.

Reviewer 02566952:

Comments for the authors

Congratulation for the meaningful work, scientific and medical practitioner community needs more of there clinical studies and publishing of long term results. For the sake of keeping the manuscript concise but still informative, I would suggest adding one or two phrases sin the introduction about current stage of stem cell based therapy in treating liver cirrhosis? Is this a common practice procedure, do the cited studies perform stem cell transplantation of autologous or allogeneic origin, differentiated or non differentiated stem cells, what type of stem cells? In special condition in the lab BMMMScs were shown to differentiate to hepatocytes, however if this happens truly in vivo or of stem cells have rather a cytokine based regeneration potentiating effect it is not yet clear. It is not clear for me from the methods description what was the exact formulation for stem cell therapy in the case of bone marrow derived source. It seems it was rather bone marrow aspirate that does contain a (very limited) amount of BMMSCs but is composed mostly of other monocytic cells of mesenchymal and endothelial origin. As the second "therapeutic branch was basically HSC transplantation, probably stem cell were obtain by apheresis. Please confirm if it is the case. Is there literature support for treating liver cirrhosis using HSCs? (non differentiated obviously) if so, please comment and cite. Results from table 1 and 3 would benefit from being shown in chart form I have a problem with this statement "bone marrow or HSCs transplantation was performed accordingly to patient willingness. First of all I am not sure it was BMMScs transplantation or bone marrow aspirate (please document) and second on what bases could the patient choose, what they were offered in terms of informed consent as this manuscript does not offer clear information about result from previous studies comparing BMMSCs and HSCs effect, their comparative advantages and caveats. How many follow up visits were performed average per time unit (month, or year, how it is the case and on what criteria) What was the therapeutic regimen (cells per ml, how many ml, vehicle, timing, frequency cy of administration?) All these parameters are known to influence SCT efficiency and safety and need to be mentioned. I would argue that there were a limited amount of true mesenchymnal stem cells in the case of bone marrow aspirate infusion. Without proper preconditioning such cells they adopt a fate that is dictated by their environment therefore if a very early tumorigenic process was already undergoing in patients at the moment of injection or

occurred early after this malignancies were only fuelled by cell cocktail administration. Maybe it is not a problem of the cell therapy in itself but of the cell type, modality of preconditioning and delivery. Moreover, all the treated cases seem to fail into the category of advanced complicated liver cirrhosis. Treated and non-treated patients seem to have similar mortality rate. As authors themselves state, it is hard to conceive that irreversible morphological and physiological alterations due to vascular and parenchimatous liver failure can be reversed by couple of circulating stem cells injected in a harsh environment. Rather the long term follow up in this study coincides with the natural history of the condition itself that might be indeed aggravated by injecting uncommitted progenitors within a tumor prone environment. Follow up of the fate of administered stem cells using combined Imagistics methods has been proposed as a method to discriminate tumorigenic transformation and should be maybe mentioned (Labusca, Herea, J NanosciNanotechnol 2018) . What is the author opinion about eventual modalities to improve performance of SCT (such as vehicle based delivery to support hepatic development and anti-inflammatory activity, use of preconditioned cells, delivery on earlier stages of the disease to more carefully selected patients). Overall this is an important information about long term follow up of cell treated patients following the mentioned therapeutic protocol and stage of the disease from which all clinical and scientific comunitu can benefit.

Response:

1. Thanks a lot for the reviewer's comments. Currently, phase I/II clinical trials have been conducted to evaluate the efficacy of stem cell therapy for treating liver cirrhosis, and in these studies, non-differentiated stem cells of autologous origin were used, which mainly included hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). To date, the most frequently studied stem cells are the MSCs. Notably, bone marrow (BM)-MSCs have been prevalently utilized. Autologous injections of BM-MSCs were reported to result in improvement of liver function, which showed significantly improved Child-Pugh and MELD scores. In addition, the transplanted cells were mostly infused intravenously. A more description pertaining to the stem cell therapy for treating liver cirrhosis has been added in the revised manuscript.
2. In this study, the time of the subjects enrollment was 10 years ago, when the BMMSCs were

harvested through multi-site bone marrow puncture due to the experimental conditions, in order to capture as many MSCs as possible, with more than 10^6 stem cells per ml captured. This approach of BMMSCs collection had been reported in previous studies. To increase the number of hematopoietic stem cells in the circulatory system, G-CSF was administered 7 days prior to transplantation to mobilize bone marrow hematopoietic stem cells into the peripheral blood, which had been proved to be feasible and effective. The collection of stem cells is usually done with a blood cell separator, and HSCs have shown effective for the treatment of liver cirrhosis. This has been described in the revised manuscript, and references have been cited.

3. Currently, BMMSCs and HSCs have been demonstrated to be effective for treatment of liver cirrhosis; however, there are no RCTs report which types of stem cells are more effective and safer for treating liver cirrhosis until now. BMMScs are captured through multi-site bone marrow puncture, which is invasive; while peripheral blood stem cells are harvested with a blood cell separator, which is less invasive. In this study, the types of stem cells used for transplantation mainly depended on subjects' willingness. Following collection of stem cells (approximately 100 ml, at a density of $(2.81 \pm 1.03) \times 10^6$ cells/ml), stem cells were infused into the liver via the hepatic artery. Follow-up was doe according to the EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis, and liver functions and imaging were re-examined once every 3 to 6 months to monitor the efficacy and tumorigenesis.

4. "Follow up of the fate of administered stem cells using combined imagistics methods has been proposed as a method to discriminate tumorigenic transformation" has been described in the revised manuscript and references have been cited.

5. Thanks a lot for the reviewer's comments. In future studies, we will modify the collection of BMMSCs and pretreatment of stem cells, or select the patients with early-stage decompensated cirrhosis (low MELD), so as to further assess the efficacy and safety of stem cell transplantation.