Dear Editor:

Thank you very much for your kind letter, along with the constructive comments from the reviewer concerning our manuscript (NO.: 65803, Minireviews). We have made modifications which are marked by underline in the revised manuscript, as well as point-by-point responses which are attached below. We believe that our manuscript has considerably improved upon revision and hope that it is suitable for publication.

Thank you for your consideration. I look forward to hearing from you.

Yours sincerely,

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Replies to the reviewer's comments:

We appreciate for your kind recommendation and criticism. Specific to your opinion, we have made modifications which are underlined in the revised manuscript and point-by-point response, as follows:

1. **In the introduction, the author mentioned three immunotherapies, but the introduction of vaccine therapy and adoptive cell transfer (ACT) is insufficient and needs to be supplemented.**

Reply 1: The major concern of our review is the immunotherapy in the setting of liver transplantation (LT) for HCC patients. Researches in this field are still at an early stage. To the best of our knowledge, there are a total of 21 papers published in reference to immunotherapy for HCC patients awaiting or after LT. Among them, 15 are about immune checkpoint inhibitors (ICIs), 6 are about adoptive cell transfer (ACT), and none is related to vaccine therapy. Therefore, the information on ACT or vaccine therapy specific to LT is limited or even absent. In the revised manuscript, we added this background in the section of *Introduction*, and supplemented more details about efficacy and safety of ACT used for HCC recurrence after LT in the section 4.1 and 4.2. Besides, we discussed the choice of ACT and vaccine therapy and prospected their further application in LT in section 5.2 of the revised manuscript.

2. **There is very little description of vaccine therapy in the article.**

Reply 2: As mentioned above, there is still no study reporting vaccine therapy in the setting of LT. Even in non-transplant setting, only few trials of vaccine therapy targeting HCC-associated antigens were performed and none of them has provided clinically meaningful results. However, a strategy using neoantigens has emerged as a promising approach to develop cancer vaccines with intense tumor-specific non-toxic responses, due to the advancements in the field of high-throughput screening. The ability to predict highly immunogenic neoantigens with anti-tumor activity as vaccines using this approach has been shown in melanoma and glioblastoma. Although vaccines were traditionally considered as a stand-alone therapy, there is a tendency to combined them with ICIs or ACT. We have added relevant description of

vaccine therapy in section 5.2 of the revised manuscript.

3. **Systematic interrogation of tumor-infiltrating lymphocytes is key to the development of immunotherapies and the prediction of their clinical responses in cancers. This needs to be discussed.**

Reply 3: TILs are a class of lymphocytes in tumor microenvironment affecting carcinogenesis, and include CD8+ T cells, CD4+ T cells, tumor associated macrophages (TAM), tumor associated neutrophils (TAN), myeloid derived suppressor cells (MDSC) and NK cells. Increased density of specific TILs phenotype, particularly activated CD8+ TILs, are correlated with small tumor size, early TNM stage and better prognosis in HCC patients. In tumor microenvironment, CD8+ TILs are in an exhausted or dysfunctional status. Failure of CD8+ TILs to kill tumor cells involves signals from multiple cells including MDSC, Treg, and TAM. The interaction of PD-L1 with PD-1 on the CD8+ TILs causes suppression and decrease in their effector function leading to decreased tumor cell death. Furthermore, the galectin-9 and T-cell immunoglobulin and mucin-domain containing (TIM)-3 interaction on MDSC and IL-10 secretion by Treg cause a similar effect. Therefore, TILs and PD-L1 should be combined to guide the development of immunotherapies, as well as predict their clinical responses in cancers. We have made a more detailed discussion on TILs in section 5.5 of the revised manuscript.

4. **Immune-checkpoint inhibitors are now being incorporated into the HCC treatment armamentarium and combinations of molecularly targeted therapies with immunotherapies are emerging as tools to boost the immune response. Therefore, it is necessary to focus on this part of the discussion (molecularly targeted therapies with immunotherapies).**

Reply 4: The FDA, EMA and other regulatory agencies worldwide have approved the PD-L1 inhibitor atezolizumab plus vascular endothelial growth factor (VEGF) inhibitor for first-line therapy in HCC. Atezolizumab plus bevacizumab is now listed as the preferred regimen in first-line systemic therapies by National Comprehensive Cancer Network (NCCN) guidelines for HCC, replacing sorafenib and Lenvatinib.

The combination with lenvatinib was associated with double the response rate compared with the response rate observed with single-agent pembrolizumab, but at the cost of increased toxicity. Currently, a number of phase III clinical trials using combination of molecularly targeted therapies with immunotherapies are being conducted. If one or more of them also show positive results, the choice of preferred treatment will depend substantially on patient characteristics, tolerability and toxicity profile, and the preferred strategy would offer concrete experience to draw upon for HCC patients in LT setting. We have discussed the combinations strategies, focusing on molecularly targeted therapies with immunotherapies, in section 5.4 of the revised manuscript.

5. **The title of the paper is 《Immunotherapy in liver transplantation for hepatocellular carcinoma: pros and cons》, but the conclusion does not highlight the main point of the paper.**

Reply 5: Within the recent decade, the breakthroughs of immunotherapy have greatly expanded the treatment armamentarium for HCC. However, there still is an unlighted corner for HCC patients awaiting or after LT, due to the deep concern about lethal rejection induced by immunotherapy. On the one hand, there will be more and more HCC patients after immunotherapy who are bridged or downstaged to be candidates for LT, as immunotherapy is now gradually becoming a part of routine or even preferred regimens for HCC systemic therapy; and there are also many patients with HCC recurrence after LT who fail to respond to other therapies and immunotherapy may be their last option. We must face the demand for immunotherapy in the setting of LT. On the other hand, the rejection rate, especially the lethal pattern, is higher than we can afford; and there are many unsolved problems when immunotherapy coexists with immunosuppressants in the setting of LT. Therefore, we need to explore immunotherapies in LT for HCC with caution, regarding immunosuppressant adjustment, biomarkers for safety and efficacy, as well as the selection strategies for different immunotherapies and patients. We have revised the conclusion to highlight the main point of the paper in concert with the title.

Thanks again for your professional review and criticism. Hope for your further recommendation.