Dear Reviewers:

Thank you very much for your valuable suggestions. And we have revised the manuscript in response to your suggestions.

Reviewer #1:

I would like to commend the authors for the diligent work. I believe the manuscript will have a significant impact to the literature. I would like to suggest minor revisions to the authors:

1. In the introduction section, the authors should elaborate on the attempts of immunotherapy in hepatocellular carcinoma and the expected benefits of such an approach. This can continue with the aims statement of the manuscript.

Authors: We have improved the manuscript according to this suggestion and made the introduction section more logical. The following content has been added: "In recent years, immunotherapy holds great promise to patients with HCC. Immune checkpoint inhibitor (ICI) therapy[3] and adoptive cell therapy, represented by anti-PD1 antibody therapy and chimeric antigen receptor T cell (CAR-T) therapy[4], have made a major contribution to HCC immunotherapy. However, there remains limitations in these immunotherapies. Anti-PD1 antibody therapy induces severe side effects in most patients and the beneficiary group is limited; CAR-T therapy has low effectiveness in solid tumors. These shortcomings limited wide clinical application of HCC immunotherapy, and new therapeutic strategies are needed to remedy these obstacles. The study of HCC neoantigens and personalized neoantigen vaccines is a promising direction. Here, we review current research related to HCC neoantigens. Specifically, we discuss the methods for screening and identifying HCC neoantigens and strategies for

exploiting HCC neoantigens in immunotherapy."

2. "…….Therefore, HCC is a cancer with a median TMB and may have fewer mutation-induced neoantigens than melanoma[10]……" this is actually a repeat statement that has been expressed before during the general information regarding neoantigens.

Authors: We have deleted the repeat statement.

3. "The above results suggest that the process used for detection of mutation-induced neoantigens in melanoma may not be ideal for HCC mutation-induced neoantigens." Regarding this statement, do the authors have a counter proposal or are there any alternative methods that have been developed.

Authors: This example illustrates that the most challenging process of HCC neoantigen immunotherapy is the screening and identification of HCC neoantigens owing to the relatively low tumor mutation burden compared to melanoma. It is necessary to optimize the process according to the characteristics (such as HBV infection) of HCC when we use it in HCC. We have an in-depth discussion in the second part of the article and a simple explanation is given here in the manuscript.

4. "In comparison, the sampling of circulating tumor cells (CTCs) in human peripheral blood is more convenient. Because of the high heterogeneity of HCC[21], more comprehensive gene information can be provided by CTCs than by tumor samples." How can we justify this statement. CTC are cells that pass to the systemic circulation through portal vein invasion. They can provide information regarding diagnosis or the prognosis of HCC but to determine neoantigens from these cells would not represent the whole tumor. It will provide an easy access rather than a core biopsy but would negative

neoantigens mean that the tumor does not produce any neoantigens or the tumor mutation burden is low?

Authors: We agree with you very much and have adjusted some of the contents. We have deleted the statement: "Because of the high heterogeneity of HCC[21], more comprehensive gene information can be provided by CTCs than by tumor samples." And we have added the statement: "A large amount of biological information such as gene mutations can be obtained from CTCs [23]".

23 **Lohr JG**, Adalsteinsson VA, Cibulskis K, et al. Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer. *Nat Biotechnol* 2014; **32**: 479-484 [PMID: 24752078 DOI: 10.1038/nbt.2892]

5. "…….that all of the predictions were failed." should be corrected as ""…….that all of the predictions had failed."

Authors: We have revised this mistake as requested.

This is an exciting area of research that should be promoted for future translational research regarding HCC.

Authors: Many thanks again for your support.

Reviewer #2:

Authors reviewed neoantigens as a promising targets in immunotherapy of HCC. This review offers some novel strategies to solve existing problems in HCC neoantigen research and provide further insights for immunotherapy.

Authors: Many thanks again for your support.

Best wishes,

Yours sincerely,

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