Peer Review File

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##Response to reviewer A

General comment. The paper titled “The combination of a prolonged treatment time window and alpha-fetoprotein benefits the tumor response of hepatocellular carcinoma patients as evaluated by the imRECIST” is interesting. In the process of immunotherapy for HCC patients, the time window for treatment may need to be extended. An analysis of AFP may assist the imRECIST by providing a more accurate evaluation of tumor progression. However, there are several minor issues that if addressed would significantly improve the manuscript.
Response. Thank you for your constructive comments on my manuscript. We have carefully considered the suggestions and have tried our best to improve and made some changes in the manuscript. Detailed point-to-point responses are given as follows.

Specific Comments
Comment1(C1). The description of some methods in this study is too simplistic, please describe in detail.
Reply1(R1): Thank you for your valuable suggestion. We have provided a more detailed description in the method (see Page6, line176). In addition, for the subgroup assignment of patients, we have provided a specific and detailed description in this section (see Page7, line215) in order to facilitate readers' understanding.
Changes in the text: Please see Page6, line176; Page7, line215.

Comment2(C2). In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.
Reply2(R2): This is undoubtedly a good suggestion. We have added relevant background knowledge and limitations of the prior study (see Page3, line72; Page4, line102), as well as the importance and clinical significance of this study in the introduction (see Page4, line118).
Changes in the text: Please see Page3, line72; Page4, line102; Page4, line118.

Comment3(C3). It is suggested to increase the research progress of molecular and immunophenotyping and combined immunotargeting therapy for hepatocellular carcinoma in the discussion.
Reply3(R3)：We sincerely appreciate your valuable feedback. The combined immunotargeting therapy is an important background component, so We have elaborated on its corresponding content operation in the introduction (see Page3, line72). We also have a detailed understanding of the research progress on the molecular and immunophenotype of HCC, and found that with the rapid development of multi-omics technologies such as genome, transcriptome, proteome, metabolome and immunotome, molecular systems for HCC have been discovered and established, and then HCC has been divided into proliferative and non-proliferative subtypes. On this basis, the subtypes with different molecular characteristics and clinical prognosis can

be found by further fine typing. Due to the numerical requirements of the article and the fit of the content to the topic, regretfully, we were unable to add this section to the article.
Changes in the text: Please see Page3, line72.

Comment4(C4). The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Hepatocellular carcinoma with complete response to the immunotherapy: the oncologist's dilemma, Hepatobiliary Surg Nutr, PMID: 352845057". It is recommended to quote this article.
Reply4(R4): It is really a very enlightening article. Thank you very much for the recommendation. After reading it, we gained a lot from it. We have already cited it in the article (see Page4, line121).
Changes in the text: Please see Page4, line121.

Comment5(C5). What is the correlation between tumor heterogeneity and immunosuppressive microenvironment in hepatocellular carcinoma? What role does the time window for treatment play in this process? It is recommended to add relevant content.
Reply5(R5): This is really an important part. In the discussion part, we have explained the relationship between the immunosuppressive microenvironment and tumor heterogeneity, and explained the role of time window in this process (see Page8, line268).
Changes in the text: Please see Page8, line268.

Comment6(C6). How to determine the criteria according to this study to screen the most suitable population for immunotherapy? It is suggested to add relevant contents.
Reply6(R6): This is a very profound question. For the results of this article, making the imRECIST more accurate in evaluating the tumor progression of patients and increasing the possibility of immunotherapy benefits for patients. For patients with SD but increased alpha fetoprotein, we should be alert to the possibility of tumor progression. Therefore, based on our current research results, we may not be able to make a final conclusion on this issue about the most suitable population for immunotherapy. But the question is so significative that it will be one of our future research goals.

Comment7(C7). How to further optimize and improve? What is the author's next research plan? It is recommended to add relevant content to the discussion.
Reply7(R7): This is a very promising issue, and there are undoubtedly some shortcomings in our research. We have listed our next research plan in the discussion section (see Page11, line349).
Changes in the text: Please see Page11, line349.

##Reviewer B

Comment1(C1). First, the title is very arbitrary because of the small sample size, the current findings are at high risk of false-negative. I suggest the authors to tone down the current title. Please also indicate the clinical research in the title such as a retrospective cohort study.

Reply1(R1)：Thank you for your valuable advice. Some modifications were made to ensure that the title not only conforms to the content, but also reflects our thinking on the current problem.
Changes in the text: Please see the title.

Comment2(C2). Second, the abstract needs some revisions. The background did not describe why the treatment time window and AFP play roles in the treatment of HCC and please briefly provide the rationale for this research focus. The methods need to describe the inclusion of subjects, the assessment of treatment efficacy outcomes, assessment of clinical factors including AFP and follow up procedures. The results need to describe the baseline clinical characteristics of the study sample. The conclusion needs to be tone down and have comments for the unaddressed questions of this study.
Reply2(R2): Thank you for your valuable guidance. Your valuable suggestions have been changed into the corresponding content added in the abstract (see Page 1, line 29). For the method section, due to the cumbersome grouping method, we have simplified the subgroup assignment in the method section of the abstract. Additionally, for the sake of readers' good understanding, we have provided a specific and detailed description for the subgroup assignment (see Page7, line 215). For the description of assessment of clinical factors including AFP and follow up procedures, we have detailed description in the methods of the article because of the limitation of the page (see Page6, line176). For the description of the baseline characteristics of the patients in the results section of the abstract, we have detailed description in the results of the article because of the limitation of the page (see Page6, line197).
Changes in the text: see Page 1, line 29; Page7, line 215; Page6, line176; Page6, line197.

Comment3(C3). Third, in the introduction of the main text, the authors did not review what the roles of treatment time window and AFP in the assessment of the treatment response of ICIs, and what the underlying mechanisms are. A review on the clinical guideline on the treatment time window and biomarkers that indicate the treatment response may be needed.
Reply3(R3): This is an important part, and we have supplemented the treatment time window and the significance of alpha fetoprotein in immunotherapy (see page4, line102). Unfortunately, the underlying mechanism is unclear, which is also the direction we will focus on and strive for in the future. In addition, the significance of biomarkers in the evaluation of treatment response has also been shortly reviewed (see page4,line107,Page 9,line 300).
Changes in the text: see page4, line102; page4, line107; Page 9, line 300.

Comment4(C4). Fourth, in the methodology of the main text, please describe the clinical research design, sample size estimation, and follow up procedures. In statistics, please ensure $P<0.05$ is two-sided. The authors need to describe the statistical comparisons of AFP levels between subgroups and adjust for the potential confounding factors since the authors focused on the relationship between AFP and treatment response transitions. It is inadequate to just describe without statistical comparisons.
Reply 4(R4): We feel great thanks for your professional review work. For the design of this study, we divided the patients into different groups, and compared their survival differences between subgroups to draw corresponding conclusions. For the sample size estimation, the

viewpoint of this study is novel, and the number of cases in our hospital meeting the inclusion criteria is small. So statistically we're not going to be perfect. Undeniably, one of the shortcomings of this study is the insufficient sample size. But we have listed our next research plan in the discussion section. In statistics, $P<0.05$ is certainly two-sided. For the statistical comparisons of AFP levels between subgroups, we compared the significance of AFP between group H (patients with SD that changed to PD with an increase in AFP and patients with PR that changed to PD with the increase of AFP) and group I (patients with sustained PD), indicating that patients with a PR or SD, but with increased AFP concentrations have a high risk of tumor progression. For the interference of confounding factors in the analysis process, there is no doubt that many confounding factors have an impact on immunotherapy. In the research process of this study, we tried our best to reduce the influence of known confounding factors on the research. Thus, there were no significant statistical differences in baseline characteristics among the all patients, even among the subgroups.

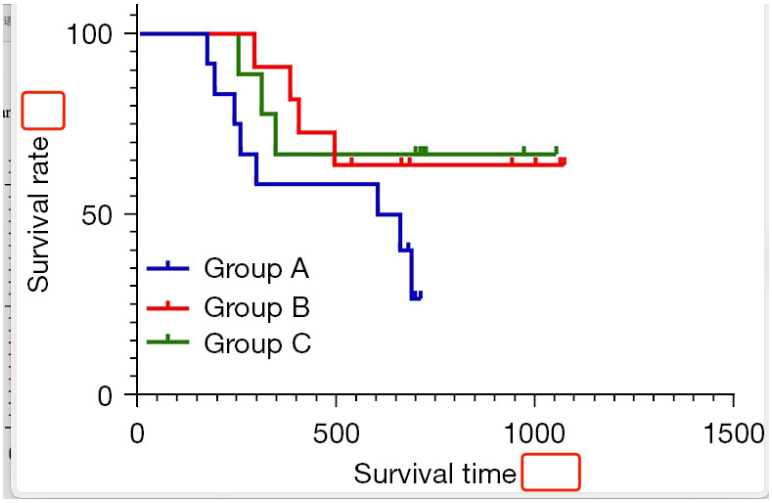
Reviewer C

1. You've mentioned "studies", while only one reference was cited in this sentence. Please check.

358 absence of such reductions, PD is likely (23, 38). In addition, studies have shown that
359 the white-blood-cell/lymphocyte ratio, neutrophil/lymphocyte ratio, and macrophage
360 level are also independent factors that can predict tumor progression after
361 immunotherapy (22). Further, some scholars have found that circulating DNA can

Reply: We apologized for such a mistake and we have added relevant reference. Of course, the following citations have also been changed accordingly.

2. Figure 1: Please indicate the units for both the X- and Y-axis.

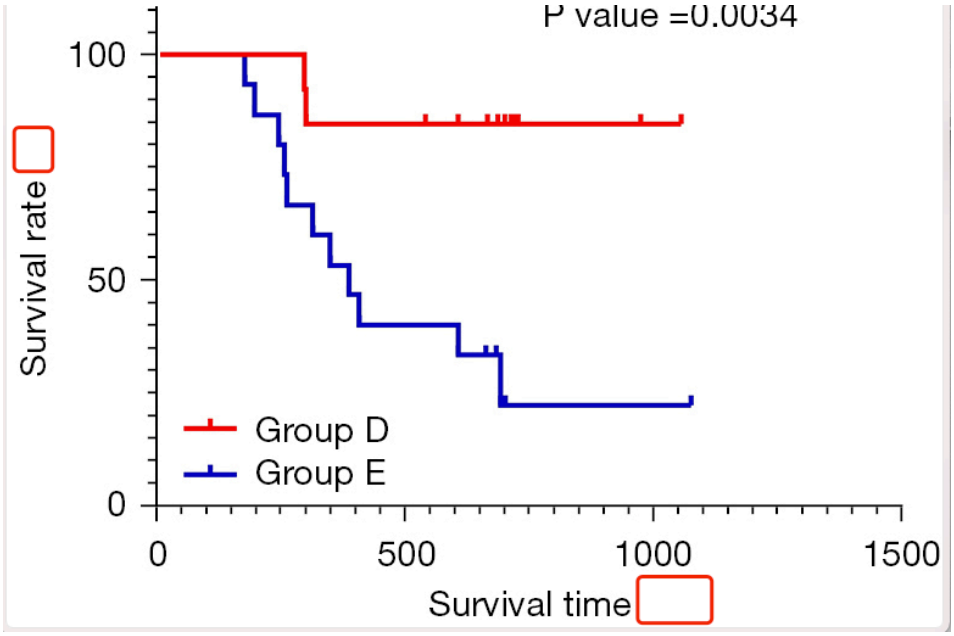


Reply: Thank you for your reminder. We have added the units.

3. Please also define PR and PD in Figure 2 legends.

Reply: Thank you for your reminder. We have added the legends.

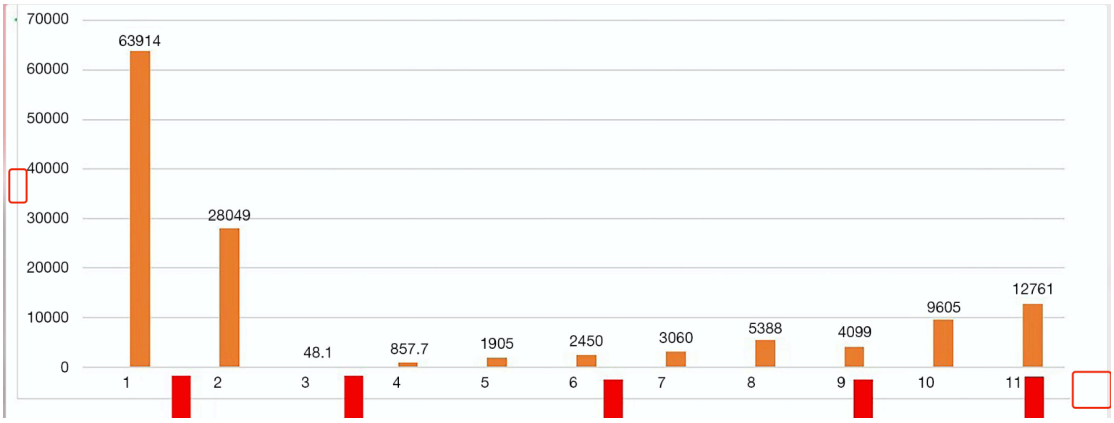
4. Figure 3-5: Please indicate the units for both the X- and Y-axis.



Reply: Thank you for your reminder. We have added the units.

5. Figure 6

a. Check if descriptions should be supplemented for X- and Y-axis.



b. Please also define CT and AFP in the figure legends.

Reply: Thank you for your reminder. We have added the units and revised the figure legends.