

18-June-2021

Editor-in-Chief, World Journal of Gastroenterology

RE: MS number 60917

Thank you very much for your review and for returning our manuscript for revision. Enclosed please find a file that contains the revised manuscript entitled “Gut microbiome composition can predict the response to nivolumab in advanced hepatocellular carcinoma patients”, as well as itemized responses to the reviewer’s comments.

***Listed below are our responses to the reviewer’s comments.**

#Reviewer: 1

Although the authors generated very interesting findings that will lead to clinical applications, I have a few minor comments below;

1) The study is limited by the small sample size. Thus, authors should be careful when proposing those potential biomarkers because they can be confounded by sample size and baseline characteristics.

Answer: The issue on the limitation by the small sample size have been described in the discussion section, in response to the reviewer’s comment as below.

A major limitation of this study is the small size of its cohort, which did not provide sufficient statistical power. This preliminary data should be interpreted with caution and further studies enrolling larger numbers of subjects may, thus, reveal additional microbial patterns. In addition, translation of a prognosis-associated microbial signature may not be straightforward, and thus several inherent variabilities between individuals within each cohort should be also considered as potential confounding factors.

2) I would suggest the authors include a paragraph indicating the strengths and limitations of the study in the discussion.

Answer: The issue on the strengths and limitations of the study have been described in the discussion section, in response to the reviewer’s suggestion as below.

A major limitation of this study is the small size of its cohort, which did not provide sufficient statistical power. This preliminary data should be interpreted with caution and further studies enrolling larger numbers of subjects may, thus, reveal additional microbial patterns. In addition, translation of a prognosis-associated microbial signature may not be straightforward, and thus several inherent variabilities between individuals within each cohort should be also considered as potential confounding factors. Nevertheless, our data highlights the promising possibility that a feasible approach may be to combine several microbial features for prediction of nivolumab treatment.

3) Fig 2A. The heatmap should include the name of bacterial taxa on Y axis.

Answer: The original Figure 2A is too large to combine with Figure 2B; however, we agree with the

importance of showing the patterns among the samples according to the treatment and prognosis. To resolve this, we have presented the original comprehensive Figure 2A, including the names of bacterial taxa, as Supplemental Figure 1. The legend of Figure 2 has been modified accordingly.

4) I would suggest using PERMANOVA tests to indicate p-values to confirm no differences for the comparisons.

Answer: We have added the statistical values, obtained using PERMANOVA test, to Supplementary Table 2. As such, the legend of Figure 2 has been modified accordingly.

5) Figure 4B. Please include the p-values in the figure for the comparisons.

Answer: Figure 4 and its legend have been revised accordingly. Please see our response to the 6th comment from Reviewer #1 also.

6) Have you compared F/B ratios as in Figure 4B?

Answer: We have compared whether there was a significant difference in the mean value of F/B ratio according to the prognosis. Although we did not find a statistically significant difference in the mean value, we did observe a skewed pattern in the *Firmicutes/Bacteroidetes* ratio (< 0.5 or > 1.5) for the non-responders. We have added this graphical data to Figure 4B.

7) Since all non-responders are infected by Hepatitis B, do you think the treatment response is associated with Hepatitis B infections? It would be good to include this in the discussion.

Answer: We noticed that there was mistake. Specifically, the designations of 'non-responder' and 'responder' were erroneously exchanged for each other in the heading of Supplementary Table 1. We have now corrected it in Supplementary Table 1.

In this study, all 5 responders and 1 of 3 non-responders were infected by HBV. However, this may reflect the relatively higher proportion of HBV-related HCC in the Korean population in general, rather than the positive impact of HBV infection on nivolumab outcome. The association of HBV in nivolumab treatment response is not clear yet. For further consideration, in the CheckMate 040 data, the efficacy and safety of nivolumab treatment in sorafenib-experienced patients with advanced HCC were comparable between the HBV-infected group and the non-infected group^[5].

We have discussed this notion in the revised manuscript. Thank you for your valuable comment.

***Listed below are our responses to the Editorial office's comments:**

3. Academic norms and rules: The authors need to provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found in the Bing search.

Answer: We have provided the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement with the revised manuscript. Thank you for your comment.

5. Issues raised:

(1) I found the authors have added the PMID and DOI in the reference list, PMCID should be deleted. Please revise throughout;

Answer: We have deleted the PMCID throughout the revised manuscript. Thank you for your comment.

(2) the authors need to respond positively to the peer-reviewer' comments;

Answer: We have provided point-by-point responses to each of the peer-reviewers comments above.

(3) The authors need to notice that in Supplementary Table 1, Responder group number in Child-pugh class A should be 3 but not 5.

Answer: We noticed that there was mistake in the Supplemental Table 1 heading, namely 'non-responder' and 'responder' were erroneously exchanged with one other. We have now corrected this in Supplementary Table 1. Thank you for catching this error.

***Listed below are our responses to the Company editor-in-chief comment**

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Answer: Thank you for your valuable review and comment.

We appreciate your reevaluation of the manuscript. We hope that the above modifications are satisfactory and look forward to publication in the near future.

Sincerely yours,

Eun Jeong Won, M.D., Ph.D.

Department of Parasitology and Tropical Medicine

Chonnam National University Medical School

42 Jebongro, Dongku, Gwangju, 61469, South Korea

Tel 82 (61) 379-2716

Fax 82 (62) 220-5370

E-mail: Parasite.woni@jnu.ac.kr