

June 18, 2016

Ya-Juan Ma
Science Editors
World Journal of Gastroenterology

Dear Editors:

Re: 25972: **“Immune checkpoint and inflammation as therapeutic targets in pancreatic carcinoma”**

Thank you very much for your letter regarding our manuscript entitled “Immune checkpoint and inflammation as therapeutic targets in pancreatic carcinoma.”

As instructed in the decision letter, we addressed the following key issues in our revised manuscript: (1) we carefully evaluated the rationale behind the therapies and added relevant descriptions, (2) we added descriptions of combination therapy with radiation and immune checkpoint therapy, and (3) we added explanations about MSI and mismatch repaired deficiency.

We are now submitting our revised manuscript with our point-by-point reply to the reviewers' comments in this letter.

We greatly appreciate your effort in reviewing our manuscript and in processing our revised version. We would also like to thank the reviewers for their valuable help in improving our manuscript.

We sincerely hope that our revised manuscript is now suitable for publication in *WJG* in its present form.

Sincerely,

Shiro Kimbara, Shunsuke Kondo
Department of Experimental Therapeutics, Exploratory Oncology Research & Clinical Trial Center,
National Cancer Center Hospital
5-1-1 Tsukiji Chuo-ku,
Tokyo 104-0045, Japan.
Telephone: +81-3-3542-2511
Fax: +81-3-3542-3815
Email: shkondo@ncc.go.jp

1. The rationale for immune checkpoint use in various trials for PDA is not clear. Does PDA normally have a high infiltration of CTLs? This is a pre-requisite for CTLA4 to work normally.

Response: In accordance with the reviewer's comment, we have added descriptions of the rationale of immune checkpoint. This rationale is common to various tumors including PAC. Furthermore, we emphasized why RAC is resistant to immune therapy. As you mentioned, low infiltration of CTL is one reason for the immunoresistance.

2. Author states Tregs may contribute to PDL1 resistance, but at the same time mentions “a few effector T cells infiltrate into the tumor tissue”. The next sentence seems contradictory to preceding two statements.

Response: Tregs and effector T cell are both lymphocyte, but have opposite functions. Tregs play a role in immunosuppression while effector T cells play a role in immunostimulation. Thus, the two sentences are not contradictory, in our opinion. To clarify our meaning, we have revised the sentence from “a few effector T cells infiltrate into the tumor tissue” to “few effector T cells, which play essential roles in immune checkpoint therapy infiltrate into the tumor tissue.”

3. What is microsatellite instability? Why does it promote immune response? How does mismatch repair mirror underlying MSI?

Response: In accordance with the reviewer's comment, we have included additional explanations of the concept of MSI. The underlying actions of the MSI and mismatch repair deficiency are not entirely equal because the mismatch repair deficiency is one mechanism of MSI. However, in the context of the manuscript context, it mirrors the underlying MSI.

4. How would combination therapy with cytotoxic regimens promote immunotherapy? What are the potential mechanisms that can be exploited – for example, has anyone looked at depletion of TAMs with paclitaxel? We encourage the authors to look into pre-clinical studies looking into the mechanism.

5. What would be the underlying rationale for evaluating combination immunotherapy in PDA? That is, if either (CTLA4 or PD1 blockade) didn't work well by itself why would

combination work better?

Response: In accordance with the reviewer's comment, we have added a rationale revealed in a preclinical study. We also explained the rationales of other treatment options, as far as we know.

6. Radiation and thermal/cryo ablation are well known to promote antigen presentation and/or improve immune response. Has there been work looking into these mechanisms to improve immunotherapy in PDA? Include and discuss.

Response: In accordance with the reviewer's comment, we have added a description of radiation and immune checkpoint therapy. As you pointed out, thermal/cry ablation is an important activator of the immune response. However, compared to radiotherapy, the combination of immune checkpoint therapy and ablation has not been well studied in clinical trials, as far as we know. Thus, we omitted a description of these ablation methods in our manuscript.

7. It is not clear how KRAS and p53 (tumor suppressor genes) contribute to inflammatory response – at least not directly. Further, how do these driver mutations affect immunotherapy? Melanoma which has shown most promise with immunotherapy has a high mutational load. Comparatively, KRAS and p53 mutant tumors (colorectal for example) do not have high mutational load. How does this affect antigen presentation in these tumors? Perhaps lack of immune response may be because of low mutational load/high self antigen recognition?

Response: In accordance with the reviewer's comment, we have added descriptions of the association between KRAS and the inflammatory response.

Whether driver mutations including KRAS are predictive markers of immune checkpoint therapy is an interesting issue. The underlying rationale comes from two hypotheses. First, tumors with high mutation burden provide more neoantigens and, therefore, they are easily recognized by the antigen presenting cells. Second, tumors with oncogenic driver mutations are associated with a low mutation burden because driver mutations have the potential to lead to oncogene addiction by themselves. ROS1 fusion is related to a low mutation burden. However, KRAS mutation is associated with HIGH mutation burden [J Clin Oncol 34, 2016 (Suppl; Abstr 9017)]. Interestingly, responses to nivolumab were observed in patients with both KRAS wild

and mutant NSCLC [J Clin Oncol. 2015 Jun 20;33(18):2004-12)].

In colorectal cancer with MSI, KRAS mutation may be associated with reduced PD-1 expression [J Clin Oncol 33, 2015 (Suppl; Abstr 3611)].

Furthermore, most PACs exhibit KRAS mutation but are resistant to immune checkpoint therapy.

In conclusion, mutation burden is different from driver genes, and KRAS may be related to high mutation burden. It is difficult to predict the efficacy of immune checkpoint therapy based only on KRAS status. The relationship between KRAS status and efficacy may depend on tumor type.

8. The contribution or research that combines CXCR2 and CXCL with immunotherapy is not discussed adequately.

Response: In accordance with the reviewer's comment, we have added descriptions of this aspect. CXCR2 inhibition may enhance the efficacy of PD-1 blockade therapy. However, no clinical trial has evaluated the efficacy of CXCR2 signaling blockade in malignant tumors.

9. The pathway to IL6 or NF-kB mediated response may involve cells that are involved in the secretion of these cytokines. The authors should discuss the role of such cells in the immune response and how this can be overcome.

Response: In accordance with the reviewer's comment, we have added descriptions of this issue raised. In PAC, the source of IL-6 is myeloid cells and CAFs. The somatostatin analog SOM230 (pasireotide) inhibits protein synthesis in activated CAFs and decreases IL-6 secretion.