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Response Letter
October 13, 2015

Dear Editors,

Thank you very much for your helpful comments and great reviews of our paper. I have re-written our manuscript based on the reviewers' comments and responded to each of them individually. Enclosed please find our point by point responses to the reviewer`s comments.

We look forward to having this paper accepted for publication.

With my kind regards.

Andrea Marie Ibrahim, MSc. and Dr. Yaohe Wang

Reviewer 1:

The manuscript by Andrea Marie Ibrahim and Yaohe Wang reviews viro-immunotherapy as a new strategy for treatment of pancreatic cancer. This is a nicely written review about an important and emerging topic. The latest data are summarized and the relevant literature is cited. I have only a few minor comments: The authors state that "CDKN2A, TP53, BRCA2 and SMAD4 which occur in higher grade lesions are also commonly found". However, BRCA2 mutations are not so common as the other three mentioned gene mutations/alterations.

Response: We have removed "BRCA2" from this sentence.

The sentence "Hanahan and Weinberg … in their infamous review", should read "famous" review.

Response: We have changed "infamous" to "famous". Apologizes for the this.

Reviewer 2

The review is well written but it deals with the all fields of immunotherapy. Therefore either you eliminate all chapters on anti CTL4 and anti PD1/PDL-1 either you change the title deleting viro from immunotherapy.

Response: This review is focused on the discussion of oncolytic virus and immunotherapy for pancreatic cancer as well as the promises of the combination of the two approaches. The combination of the two approaches has been recognized as the future for cancer treatment. The reason for writing "immunotherapy" and not just

immune-checkpoint therapy in the title was for the cancer vaccines and other immunotherapy approaches that are discussed as combinatory approaches as the future for pancreatic cancer treatment. However, the emphasis is indeed combining TOVs with immune checkpoint inhibitors, which is the future for cancer treatment and belonged to immunotherapy, therefore we would like to still keep the sections on anti-CTLA-4/PD-(L)1 and the original title.

Minor comments: 1) The introduction with a discussion of the limits of the traditional therapeutic approach in pancreatic cancer is too long (4 pages, 1 figure, 35 ref). Please shorten it

Response: After re-reading our introduction, we believe that all of the information is necessary to emphasis the need for a completely novel strategy to treat pancreatic cancer. Also, the background on the tumour microenvironment is also needed to understand later concepts in the review (i.e. breaking immunological tolerance in the tumour bed). Furthermore, as it is a review article, we wanted there to be a thorough background of the disease as well as the standard treatments. This will be much easier for potential readers to quickly pick up all the information about the treatment of pancreatic cancer. There is no figure associated with the introduction; the only figure in this paper is associated with the core concept, combining TOVs with immune checkpoint inhibitors. It was referred to in the introduction to show the few infiltrating T cells and overall tumour stroma in PDAC.

2) In anti CTLA you should mention the phase 1 study with tremelimumab in pancreatic cancer (Annals of Oncology 25: 1750–1755, 2014)

Response: Thanks for this suggestion. We have included this study in the CTLA-4 section in our revised manuscript.

Reviewer 3:

This manuscript reviews about the current treatment strategy for pancreatic cancer mainly focusing on the combination therapy of oncolytic virotherapy and immune checkpoint blockade agents. The manuscript is well written full of cutting edge information for the treatment strategy of pancreatic cancer. However, there are several comments which further strengthen the manuscript. Firstly, I wonder the association between TOVs and enhanced immune responses induced by anti-immune checkpoint agents is the two edged sword. The immune responses would be increased by these agents,

while at the same time the TOVs by themselves would also be affected or cancelled by these agents through blocking infection into the tumor cells or possible direct targets by the enhanced immune cells or antibodies overcoming immune tolerance. The authors should comments to these problems utilizing these new agents.

Response: We agree that this could be a double-edged sword. We have added a comment to this: "It is important to note that not only the patient's existing immune system may impede successful TOV therapy, but that the enhanced antitumour response by combinatory approaches (i.e. the inclusion of immune-checkpoint inhibitors) may also impede successful TOV infection, spread and engagement of the immune system. This stresses the importance of determining strategic combinations, dosing and timing schedules in future studies." This falls under the immunological barriers to effective treatment with TOVs, thus we feel that this may be sufficient.

Secondly, the authors describes hurdles for the oncolytic virotherapy only from the point of view of immune escaping or immunological tolerance. Other possible mechanisms which hampers oncolytic virotherapy at the present situation should also be commented in this review manuscript.

Response: This review is focused on the oncolytic virus and immunotherapy as well as the combination of the two strategies. We had also discussed barriers involving systemic administration such as hepatic and splenic sequestration, and that the location of the pancreas itself is a barrier to successful intratumoural delivery. We focused on the biggest hurdle, which will always be the patient's immune system. Again, this is specific to PDAC, where the TME composition is really thick and heterogeneous, thus impedes successful drug delivery, and perhaps infection spread. In addition, we had also included a recent study proposing that CAFs lead to increased susceptibility to infection by TOVs while increasing their spread, and as a result enhancing antitumour immunity. This also relates to your last comment, whereby enhancing the antitumour response with one agent, i.e. the immune checkpoint antibodies, may impede the effect of TOVs. Determining the appropriate timing schedule is a future step required to avoid this obstacle, which was added in the conclusion section: "As this combinatory approach may exist as a double-edged sword, it is crucial to determine appropriate timing, dosing and sequence schedules of each agent".