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Jia-Ping Yan, Director,
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Dear Director Yan,

Old manuscript ID 63714. T cells in pancreatic cancer stroma.

Firstly, we would like to thank the Reviewers for their relevant and constructive comments. We attach a revised version of the manuscript with tracked-in-red changes in the body of the text. Please find below the point-by-point responses to the Reviewers' comments in blue. Changes in the manuscript are highlighted in red. We ensured that the revised manuscript complies with WJG Author Guidelines and followed the editorial board changes for reference citation improvement.

Further to our correspondence with you on 8th April 2021 we have realised a few things:

1. Your reminders were filtered out of our Inbox to Junk Mailbox because of the University stringency on automated emails from systems such as the one you employ. We never received the reminders. Our University email filters will block further emails from any automated system.
2. We can check progress of manuscript of your online submission system by 'View Detail' function, which we had not used previously. We will use this to track manuscript progress in future and circumvent the problems associated with Point 1 above.
3. We saw deadline from your email dated 31st March and promptly made corrections. We submitted using UK time as deadline, but I think you were using China time as deadline and we missed the deadline by two hours. We were not aware of time zone differences.
4. This manuscript along with two other manuscripts form a triumvirate of related reviews which would be good to publish together. We believe the B cells in pancreatic cancer stroma (63715) has met similar fate to T cell in pancreatic cancer stroma (63714) and NK cell in pancreatic cancer stroma (63639) is still under review.

We hope you will be able to publish all three reviews together based on the excellent reviews received for our state-of-the-art articles. We are submitting this manuscript as a new submission as per your instructions. We remain at your disposal. We look forward to hearing from you at your earliest convenience.

Hemant Kocher on behalf of all co-authors. 9th April 2021

Queries and comments to improve the manuscript:

The 5-year survival of less than 8% is probably true in UK but not in other countries, in addition reference 1 is a bit outdated.
Response: Thank you for this comment. The survival information has been updated.
Revised text: Pancreatic ductal adenocarcinoma (PDAC) is a highly devastating disease with a dismal 5-year survival of less than 5% in patients with metastatic disease [1],
Updated reference 1:

In the immune landscape, the authors present the role of KRAS in driving the inflammatory reaction and recruitment of other immunosuppressive myeloid and lymphoid subsets. It would be interesting to known how other mutations, found at various stages of pancreatic cancer progression, complement immune dysfunction ultimately resulting in an immunosuppressive microenvironment.
Response: Thank you for this comment. We added further information about how other common mutations contribute to the PDAC immunosuppressive micreonvironment.

Updated Text: At early stages of cancer development, oncogenic *KRAS* expression in pancreatic cells results in the formation of pancreatic intraepithelial neoplasia (PanIN), and drives an inflammatory reaction that modulates the recruitment and infiltration of immunosuppressive myeloid and lymphoid cell subsets. *KRAS*-mutated pancreatic cells regulate the maintenance of immunoregulatory microenvironment by inducing the release of IL-6, IL-10 and transforming growth factor (TGF-β) cytokines. In the setting of sustained chronic inflammation, PanIN progression to malignant lesion is accompanied by mutations in genes such as TP53, CDKN2A and SMAD4 frequently, which further contribute to shape the immune microenvironment. For example, the mutant tumour suppressor gene TP53 are implicated in sustaining the tissue damage and chronic inflammation by enhancing the expression of NF-kB, secretion of vascular endothelial growth factor (VEGF) and activation of fibroblasts. Decreased infiltration of T and B cells and elevated numbers of Tregs were significantly correlated with CDKN2A mutations while SMAD4 mutations are involved with enhanced invasion, metastasis and immunosuppressive effects of TGF-β on immune response. [9]

What is the status of T cells in pancreatitis, a known precursor of pancreatic cancer? Is there any correlation with the progression to pancreatic cancer in the 5% of pancreatitis subjects that develop it?
Response: This has been addressed

Updated Text: Similar to PDAC, in inflammatory conditions of the pancreas, such as pancreatitis, the inflammatory reaction leads to the infiltration of myeloid cells, such as monocytes and neutrophils. Although macrophages comprise a significant population within the inflamed pancreas, T cells are also present, and infiltration of $CD4^+$ T cells has been implicated in the progression of acute pancreatitis in mice. [27] As pancreatitis progresses, the ratio of $CD4^+$ and $CD8^+$ T cell increases, with increased numbers of immunosuppressive Tregs observed in patients with chronic pancreatitis.[28]

Although the authors have provided a good figure at the end, a table comparing the normal function of the various types of T cells to their roles in pancreatic cancer setting would help the reader to a better understanding.
Response: Thank you for this comment. We added a table of T cell phenotype and functions as suggested:
New table 1: T cell phenotype and functions

The authors have documented the current therapies and the associated problems very well. Are there any ongoing clinical trials worth mentioning that are targeting the T cells as a part of the pancreatic cancer therapy?

Response: We thank the reviewer for these positive comments on our work. Alongside with the summarised strategies targeting pancreatic cancer, we updated the text with further information.

Updated text: The vast majority of trials targeted towards T cells in pancreatic cancer are centred around the use of immune inhibitory receptors against PD-1 and CTLA-4.[74] Most of these trials have enrolled patients with metastatic or borderline resectable pancreatic cancer and assessed the response to either single or double agent immunotherapy or combination therapy with chemotherapy/radiotherapy. The results regarding progression free survival or overall survival have been so far underwhelming.[75] In a meta-analysis on checkpoint inhibitors overall survival and progression-free survival showed no improvement in single agent therapy but a small number of studies on combination therapy have been more promising.[76] It is feasible that the limited tumor mutational burden of pancreatic cancer compared to immunotherapy responsive tumours, such as melanoma or non-small cell lung cancer, may be the key differentiating factor. The phase II KEYNOTE-185 study trying to assess the efficacy of pembrolizumab on patients with non-colorectal microsatellite unstable/mismatch repair deficient cancers enrolled 22 patients with pancreatic cancer, of which four patients showed response to treatment with increase in progression-free survival and median survival.[77] These results, although encouraging, demonstrate that there key barriers around identifying correct groups of patients that would benefit from T cell targeted therapies.

Figure 1 is not mentioned in the text.

Response: Thank you for pointing out our error which have now been rectified.

Updated text for Figure 1: Spatial localisation and T cell interactions within the PDAC tumour microenvironment are shown in Figure 1.

Since the authors highlight how the cancer microenvironment affects T cells in PDAC, are there studies indicating how targeting the stroma improves the T cell function in PDAC patients? If so, can this approach be combined with T cell-based therapy to yield better results?

Response: additional information is now provided.

Updated text: A variety of preclinical studies highlighting the influence of PDAC stromal components on T cell anti-tumour responses provided rationale for the development of clinical trials incorporating combined approaches to enhance T cell responses.[87] CXCL12 from cancer-associated fibroblasts synergizes with anti-PD-L1 blockade resulting in activation of T cells and tumour regression in mice.[6, 88] Similarly, dual blockade of TGF-β and anti-PD1 resulted in increased T cell responses and tumour regression [89] , targeting of myeloid cells with CSF1R in combination with PD-1 or CTLA-4 blockade [90] , CCR2 inhibitors [91] or focal adhesion kinases (FAK) inhibitors has been shown to decrease infiltration of suppressive myeloid populations with concomitant activation of T cells, and improved survival in mice models. [92]