Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-2762a

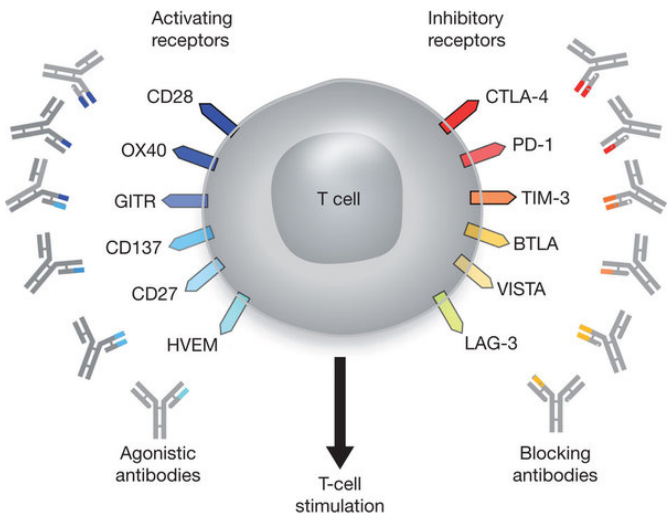
Reviewer A

The manuscript titled "Prognostic Value of Tumor Infiltrating Lymphocytes Combined with PD-L1 Expression for patients with Solitary Colorectal Cancer Liver Metastasis" is focused on studying the prognostic value of CD8+ TILs and PD-L1 expression in a subset of CRC patients with liver metastasis who underwent R0 resection. Authors found that patients with higher CD8+ TIL density and lower tumor PD-L1 expression had a prolonged RFS and OS rates. It was proposed by authors that CD8+ TIL density and PD-L1 expression can be utilized as predictors for SCRCLM clinical outcomes and determine the need for postoperative therapy. The manuscript is well-written, and the justification for patient's selection was well-explained. However, the comments below need to be addressed to improve the manuscript.

Major comments:

- Why authors only focused on CD8+ TILs and PD-L1 expression? How about tumor-infiltrating Tregs and other ICs/ligands- they have also been implicated in the prognosis of cancers-page 4

Reply: As stated in the Introduction section, CD8+ T cells are cytotoxic lymphocytes that can directly kill cancer cells in the tumor microenvironment, and they are the most studied immune cells in the investigation of the relation between TIL and patient outcome, showing associations with improved survival across a variety of cancers. On the membrane of CD8+ T cells, there are both activating and inhibitory receptors (See figure below). Among them, PD-1 has attracted much attention in recent years. The interaction between PD-1 and PD-L1 serves as a brake on CD8+ T cell activity. The tumor microenvironment is so complex that it is almost impossible to take account of all immune cells and relating cytokines in one study. Therefore, we picked the most typical one in each side—CD8+ TIL in the anti-tumor camp and PD-L1 in the pro-tumor camp. Moreover, since immunotherapy with PD-1 inhibitors has achieved great success in many cancers, we hoped that, by revealing the relation between PD-L1 expression in the tumor microenvironment and patient outcome, our study could shed additional light on the mechanism of this therapy.



From: Mellman et al, Nature Vol 480, 22: 29 Dec 2011

- Are there are any references to support the scoring system used to quantify PD-L1 expression? Page 5

Reply: Semiquantitative assessment is a widely used method in immunohistochemistry. It can be used to assess the expression of almost any proteins. The scoring system described in our study is a brief summary of the protocol (See reference below).

Changes in the text: We added the following reference to the text in the Section Methods "…and was scored by a semiquantitative method, according to the percentage and intensity of positively stained cells.[18]"

18. Taylor, C. R., and Richard M. Levenson. "Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment II." Histopathology 49.4 (2006): 411-424.

- From the methodology section, authors described that PD-L1 expression was detected in tumor tissue samples, without any specificity to cell type. On page 7, however, author stated "PD-L1 monocytes"- This is confusing and should be clarified.

Reply: Thanks for the reminder. We've replaced "PD-L1 monocytes" with "PD-L1+ cells".
Changes in the text: in the Section Results "When CD8+TIL and PD-L1+ cells were combined, 'Strong', 'Mild', and 'Weak 'subgroups…"

- RFS and OS rates for combined strong and mild CD8-PD-L1 subgroups did not reach the median (i.e. less than 36 months), however, ROC analysis showed that combined CD8-PD-L1 has a greater ability to predict recurrence after R0 resection for CRCLM than CD8 TIL or PD-L1 alone. The discrepancy of both results was not discussed? Page 7-8

Reply: Although neither the strong nor the mild CD8-PD-L1 subgroup reached median RFS and OS, the two subgroups differed significantly in the 3-year rates of RFS (89.5% vs 71.7%) and OS (93.8% vs 81.8%), as reflected in Figure 2. The more the KM curves separate from each other in the three CD8-PD-L1 subgroups, the greater the AUC will be. Therefore, in Figure 3, CD8-PD-L1 had slightly greater AUC than CD8 TIL or PD-L1 alone. We thought this discrepancy was just reflections of different aspects of statistical analyses, not contradictions that required further discussion.

Minor comments:

- Full-stops should become before the references in all the sentences throughout the manuscript.

Reply: We've revised the formats as advised.

- The title for each result should be more informative.

Reply: We added more information to these subtitles.
Changes in the text:
the second subtitle of Section Result was changed to "Clinicopathological features among CD8, PD-L1, and CD8-PD-L1 subgroups";

the third subtitle of Section Result was changed to “Comparisons of survival and predictive performance”.

- Table 3 should have headings for the columns.

Reply: Table 3 did have headings for the columns, but the table was too long and the headings were not carried over to the second page. We have formatted the table so that it fits into one page.

Reviewer B

1. The paper is organized in a clear and easy to understand manner, but the Abbreviation ‘’TIME ‘’ should be explained in the section ABSTRACT.

Reply: Thank you. We have replaced “TIME” with “tumor immune microenvironment” in the section ABSTRACT.

2. The procedure of immunohistochemical technique and patients’ classification were described in detail. However, The CD3 and CD8 staining were rather immunofluorescent than immunohistochemically.

Reply: We have changed the subtitle “Immunostaining” to “Immunofluorescent and Immunohistochemical staining”.

3. The authors used several statistical tests that ensure the reliability of the results.

Reply: Thank you!

4. The tables and the figures are appropriate, but authors should edited the tables in way that allow to hold all result in the one page (table 2 and 3). Additionally, some data were missed e.g. in ‘’Time of metastasis’’- p-vaules were incomplete.

Reply: We have formatted the tables so that they fit into one page.

Changes in the text: in table 3, the p-value of “Time of metastasis” was changed from “0.8” to “0.800”.

5. The disscusion is consequence and the references are selected in a good method.

Reply: thank you!

6. The cited papers are relevant for the analyzed topic.

Reply: thank you!

In my opinion the analysis has been done correctly. The study is interesting according to the clinical point of research.