Reviewer's report

Title: Tissue-Infiltrating Lymphocytes Signature Predicts Survival in Patients with Early/Intermediate Stage Hepatocellular Carcinoma

Version: 0 Date: 21 Dec 2018

Reviewer: Naoto Fujiwara

Reviewer's report:

General comments

This study established a new immune cell signature based on IHC for HCC and showed that a score consisting of the immune cell signature and clinical variables, named immune-clinical prognostic index or ICPI, is predictive of survival after HCC resection. Given the importance of immune-oncology in HCC, this study may be a clue to explore new strategies to treat HCC. It is very attractive that the cohort contains the large number of samples. However, the reviewer thinks that there are several major concerns in this paper.

Major comments

1. To show the usefulness of ICPI, authors compared it with the traditional staging systems. However, these staging systems are basically determined by pre-operative variables to decide treatment strategy and physicians can obtain more information on tumor status from post operation specimens such as presence or absence of microvascular invasion and so forth. Therefore, its superiority might be nothing special. ICPI should be compared with post-operative variables or be adjusted for these variables by multivariable analysis, assuming that the post-operative variables are more predictive. Importantly, approximately 10% of patients underwent a surgical treatment in spite of intermediate stage (BCLC B). This strong selection bias underestimates the predictability of intermediate stage. Authors should highlight this limitation in Discussion.

2. The comparisons should be mainly performed in validation cohort. At least, analyses in validation cohort should be shown in the main figures. The comparisons in training cohort generally tend to overestimate the newly developed predictor/score. In addition, statistical analyses are needed to show the superiority, including p values.

3. Is TRIS associated with clinicopathological variables such as tumor size or histological variants? The reviewer thinks that the summary of these associations is very informative.
Recently, steatohepatitic HCC, one of histological variants which is characterized by intratumoral immune cell infiltrate, has been paid attention to because of increasing number of NASH-related HCC. If authors can show the impact of different types of intratumoral immune cells or immune cells in the adjacent liver on prognosis in patients with different variant of HCC, many readers would be interested.

4. A recent paper showed that intratumoral tertiary lymphoid structure is associated with low risk of HCC recurrence after resection (Calderaro et al., J Hep 2018). On the other hand, another paper showed that ectopic lymphoid structure in the adjacent liver is a microenvironment of HCC initiation (Finkin et al., Nature Immunology 2016). Therefore, that will be interesting if authors investigate not only quantity of immune cells but also their histological structure.

5. Overall, description of method is insufficient. Please add the description about how authors obtained the IHC data from TCGA in Figure 1C? In Page 6, line 52, authors defined the level of immune markers as the number of positively stained cells. However, tissue may have blank area like Patient 11 in Fig. S2. How were these blank areas adjusted? What is the statistical method to compare the survival curves? Authors should update the Methods section more precisely.

Minor comments

1. In the Abstract, subscripts of T or P with immune cell markers should not be used because there is no explanation.

2. Is this a prospective study or retrospective study? If retrospectively analyzed, why did authors use Dec 1. 2011 as the censor date (7 years ago)?

3. In page 4, why are CEA and CA19-9 used for regular follow-up after HCC resection? To my knowledge, there is no recommendation to use them in the guidelines.

4. In page 5, line 2, the initials of the two reviewers should be described. I guess that they are listed as co-authors.

5. In page 5, line 44, what does "(tumor and peritumor)"?

6. In page 6, line 17, what kind of statistical method was used?

7. In Figure 1C and D, the same color scale should be used.

8. In Page 6, line 39, what is the last cluster?

9. In Page 6, line 42, LASSO Cox regression model paper should be cited.

10. In Page 7 line 8, the definition of tumor differentiation should be described with the citation. Edmondson-Steiner grade?
11. In Page 7 line 44, the citation of X-tile program is required in the main text.

12. In the Results section, all variables which were explored in univariable analysis should be described in Table 2 to know the validity of the analyses.

13. In Table 1, sum of percentages of etiology in validation is more than 100%.

14. Figure 4A is difficult to understand. What is the message from this figure?

15. Figure 4C is the expected result because the levels used here are factors of ICPI.

16. In Figure 4D, images for each score is from the same patient? Also, images of CD68 should be added.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I recommend additional statistical review

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