Peer Review File

Article information: https://dx.doi.org/10.21037/tgh-23-61

Reviewer A

Wu and the colleagues proposed to use TCR diversity to predict the clinical outcome for GI cancer patients. My major concerns are the sample size is too small which includes 3 different cancers. External validation or at least internal validation is expected. There is no clinical or biologically interpretation of the DE50 score which is the main predictor.

Reply:

Thank you for the insightful comments from the reviewers, with which we are in complete agreement. Indeed, when we calculated the sample size, we taking into account the effect size seen in similar studies. (Cell Rep Med. 2020;1(8):100141; Cancer Immunol Res. 2019;7(1):77-85.) We have also acknowledged the constraint of the small sample size in the Discussion section of our manuscript. Additionally, the clinical implications of TCR diversity are set to undergo further exploration in a single-arm phase 2 clinical trial conducted by our team (ClinicalTrials.gov Identifier: NCT04503967). Consequently, we remain confident in the validity and the significance of the current study, believing it would be helpful to the readers, notwithstanding the limited sample size.

Changes in the text:

According to the reviewer's suggestion, internal validation was done for the prediction model **(see Page 12, line 254-257; Fig. S5).** The biologically interpretation of the DE50 score have been clearly documented in "Abstract" section and "Results" section **(see Page 2, line 27-29; Page 10, line 208-212).**

Minor comments:

1. Abstract: What is DE50 score and its relationship with TCR profiling? Should mention in the abstract. What is PLR? GIC?

Reply 1: We thank the reviewer for this insight. The DE50 score and its integral association with TCR profiling are pivotal to our study, and we appreciate the reviewer for highlighting the need for their inclusion in the abstract. Consequently, we have elucidated the clinical implications of the DE50 score in conjunction with TCR profiling within the abstract to provide comprehensive insight. Furthermore, the abbreviations PLR and GIC have been defined in the abstract.

Changes in the text: We have modified our text as advised (**see Page 2, line 27-29 ; Page 2, line 22,36**).

2. Need clearly define PFS, from which date to which date.

Reply 2: Thank you for the reviewer's suggestion. We have delineated the term PFS

regarding the timespan and parameters within the "Patients" section of our manuscript.
Changes in the text: We have modified our text as advised **(see Page 5, line 101-102).**

3. Statistical analysis: Statistical significance was calculated by two-sided t-test (95% confidence interval). This is incorrect, because as written in the rest of the paragraph, some other tests were used. The correct statement should be "Statistical significance was declared based on Pvalue < 0.05 with two-sided test."
Reply 3: Thank you for the reviewer's suggestion. Acknowledging the insightful feedback from the reviewer, we concur that our initial representation of the statistical analysis was indeed imprecise. We have rectified this in " Statistical analysis" section to accurately reflect that statistical significance was determined based on a P-value < 0.05 with a two-sided test.
Changes in the text: We have modified our text as advised **(see Page 7, line 150-151).**

4. Statistical analysis: Correlations between variables, such as TCR repertoire diversity, curative effect or adverse events, were analyzed using Mann-Whitney U test. This is also not appropriately stated. Correlation is always between two continuous variables. The correct way to state is that "To compare the continuous variable, such as TCR repertoire diversity, between groups (e.g. curative effect or adverse events), Mann-Whitney U test was used."
Reply 4: We are grateful to the reviewer for their discerning observation. We recognize that the initial phrasing was indeed ambiguous and potentially misleading. Subsequently, we have refined the sentence to accurately state.
Changes in the text: We have modified our text as advised **(see Page 8, line 152-153).**

5. Statistical analysis: Independent predictive factors for clinical efficacy were investigated by univariable and multivariable logistic regression models. This can only be assessed by multivariable logistic regression models, not univariable.
Reply 5: We thank the reviewer for this insight. We acknowledge that our initial description regarding the utilization of both univariable and multivariable logistic regression models was inaccurate. Accordingly, we have revised it.
Changes in the text: We have modified our text as advised **(see Page 8, line 153-154).**

6. Statistical analysis: According to the P-value of the outputs from the univariable logistic regression models,… What is the actual P-value criteria?
Reply 6: We appreciate the reviewer's attention to detail and their request for clarification. We have accordingly specified in our manuscript that the actual criterion for the P-value in question was < 0.05.
Changes in the text: We added the actual P-value in "Statistical Analysis" section **(see Page 8, line 157).**

7. Statistical analysis: ***p-value < 0.001, ** p-value <0.01, * p-value < 0.05. Should be included in the figure legend, not the method section.
Reply 7: Thank you for the reviewer's suggestion. We have subsequently incorporated

the denotations ***p-value < 0.001, ** p-value <0.01, * p-value < 0.05 directly into the figure legend as advised.
Changes in the text: We have modified our text as advised **(see Page 19, line 419).**

8. For all PFS K-M curves, please provide median PFS within each subgroup.
Reply 8: We totally agree with the reviewer's insightful suggestion. The median PFS within each subgroup has been added for all PFS K-M curves.
Changes in the text: We have modified our Figures as advised **(Figure3;Figure 7).**

9. Instead of providing descriptive V and J gene information, the formal VJ gene usage analysis should be performed. For example, He T et al. Novel Ensemble Feature Selection Approach and Application in Repertoire Sequencing Data. Front Genet. 2022 Apr 26;13:821832. doi: 10.3389/fgene.2022.821832. PMID: 35559031; PMCID: PMC9086194.
Reply 9: We totally agree with the reviewer's insightful suggestion. Accordingly, supplementary figure 4C and the associated text have been added to the analysis for the formal VJ gene usage.
Changes in the text: We have modified our figure and text as advised **(see Page 10, line197-201; Fig. S4C).**

10. Figure 1: Since evenness is defined based on Shannon index, should put Shannon index first.
Reply 10: We totally agree with the reviewer's insightful suggestion. Accordingly, Figure 1 and the associated text have been revised to position the Shannon index before the introduction of evenness.
Changes in the text: We have modified our figure and text as advised **(see Page 10, line 204-208; Figure 1).**

11. I don't see the necessary to include irAE in the paper as I could not draw the conclusion as stated in the manuscript that "These results suggested that the DE50 scores might contribute to slightly higher risk of irAEs and improvement in PFS with anti-PD-L1 therapy."
Reply 11: We fully acknowledge reviewer's perspective. Given our evaluation of the research data, we agree that the correlation between irAE and DE50 scores is tenuous, rendering the conclusions unreliable.
Changes in the text: Figure 3 and discussions regarding irAE have been omitted from the manuscript.

12. Not sure how the validation was done for the prediction model.
Reply 12: We totally agree with the reviewer's insightful suggestion. Accordingly, we describe how to construct and validate our prediction model in " Statistical Analysis" section.
Changes in the text: We have modified our figure and text as advised **(see Page8, line 168-173).**

13. DE50 was the primary predictor, however, there is no explanation or interpretation of DE50 score.

Reply 13: We totally agree with the reviewer's insightful suggestion. As the primary predictor, DE50 score is very important in our study, and the interpretation of DE50 score has been clearly documented in "Results" section.

Changes in the text: We have modified our text as advised **(Page 10, line 208-212)**

14. Discussions are too lengthy, need to be shortened to focus on the current study.

Reply 12: We extend our gratitude to the reviewer for their constructive suggestion. We are in complete accord with the reviewer's advice. As a result, the discussion section has been refined and concentrated to emphasize predominantly the findings and implications of the current study.

Changes in the text: We have modified our text as advised **(see Page 13, line266-268).**

Reviewer B

1. Ethics:

   1) The patients are from "Zhongshan Hospital", not "Fudan University Shanghai Cancer Center". And no author is from "Fudan University Shanghai Cancer Center" in the Title page. Please unify the institution name in your manuscript.

   2) Please provide the approval number of IRB in the Methods and Footnote sections.

138 Declaration of Helsinki (as revised in 2013) and approved by the Institutional Review Board of Fudan

139 University Shanghai Cancer Center (approval number: XXX). All patients signed the written informed

140 consents before participation.

Reply: We thank the reviewer for this insight. We have modified our text (**see Page 7, line124-125; Page 17, line 340-341**).

2. Figures and table

   1) Legend for Figure 1A and 1B should be swapped. Please check and revise.

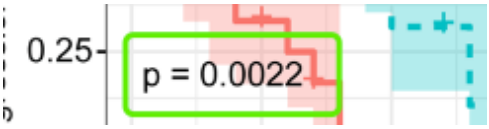
   - (A) Evenness value; (B) Shannon diversity;

Reply: We thank the reviewer for this insight. We have modified our text as advised **(see Page 21, line 455-456).**

   2) The p value is inconsistent with Figure 3C.

293 respectively) (Fig. 3A-B). However, patients with higher baseline DE50 had significantly longer PFS

294 than patients with low baseline DE50 (p=0.022) (Fig. 3C). Thus, in order to assess the independent

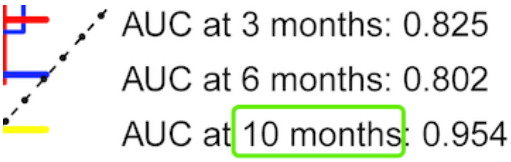


Reply: We thank the reviewer for this insight. We have modified our text as advised **(see Page 12, line 242).**

3) It's suggested to add units for the variables in Figures 2-4.
Reply: We thank the reviewer for this insight. We have modified our Figures as advised (**see Figures 2-4**). Additionally, some of these parameters including Sex, Evenness, Shannon diversity, DE50, NLR and PLR, have no units.

4) Please remove **(%)** in the Y-axis in **Figures 3 and 7A** as the rate is 0-1.
Reply: We thank the reviewer for this insight. We have modified our Figures as advised (**see Figures 3 and 7A**).

5) Figure 7B: it should be "12 months".



Reply: We thank the reviewer for this insight. We have modified our Figures as advised (**see Figure 7B**).

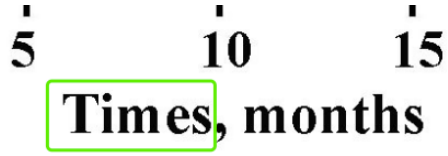
Table 1

6) Please remove "%" in all data as the table head has mentioned it.
7) Please add a unit to "Age".
8) Please define "GIC" "ECOG" "PD-L1" "CEA" "CPS" in table 1's footnote.
9) Please check the percentage of below item.

PD-L1 agents	
22C3	8 (62%)
E1L3N	5(38%)

Reply: Thank you for the reviewer's suggestion. We have modified our table as advised **(see table 1)**.

3. Supplementary Figures
1) Please provide **legends for Figures S1-S5** in the manuscript.
2) Figure S3: it should be "Time".



Reply: Thank you for the reviewer's suggestion. We have modified our text and figures as advised **(see Page 22, line 476-490; Figure S1; Figure S3)**.

4. ***Ref 12*** and ***Ref 24*** are duplicated, please check and revise.

Reply: We thank the reviewer for this insight. We have modified our text as advised **(see Page 19, line 402)**.