**Peer Review File**

**Article information:** https://dx.doi.org/10.21037/tau-23-21

**Review comment-reviewer A**

**Comment 1:** First of all, my major concern regarding this study is the problematic focus of this study, the mutation classifier for predicting the efficacy of ICIs, because the authors did not report the predictive accuracy of the classifier, rather they only tested the prognostic role of the classifier by using HR and P values, no predictive accuracy data in the validation sample such as AUC and sensitivity. A further methodology problem is no adjustment of other clinical covariates when analyzing the prognostic role of the risk score. The authors need to revise the paper substantially from title to the discussion.

**Reply 1:** Thank you very much for the constructive comment. We apologize for our evaluation of the prognostic role of the classifier by only using HR and P values. We have included ROC curve analyses in predicting the overall survival in ICI therapy cohorts (the CheckMate ICI therapy cohort, the MSKCC advanced RCC ICI therapy cohort and the MSKCC pan-cancer ICI therapy cohort). We have further taken adjustment of other clinical covariates (such as the information of gender, age and TMB that we could collect) using multivariate Cox regression, and evaluated the prediction effect of the classifier by C-index.

**Changes in the text:**

We have added the results of ROC curve analyses and C-indices (see Page 11, line 340-348; Page 12, line 363-365; Page 13, line 395-401; Figure S4 A-C; Figure S4 E-F).

**Comment 2:** Second, the title did not indicate the research design, i.e., the development and validation of a predictive model.

**Reply 2:** Thank you for your valuable suggestion. We have revised our article title.

**Changes in the text:**

We have modified our article title as advised (see Page 1, line 3-5).

**Comment 3:** Third, the abstract needs some revisions. The background did not have comments on the knowledge gaps and limitations of prior studies on the efficacy prediction of ICIs and explain why there is need to develop a predictive model based on genes. The methods did not describe the clinical factors and efficacy outcomes in the databases and how the training and validation samples were generated, as well as the statistical methods for assessing the predictive accuracy. The results need to quantify the prognostic role of the risk score by using HR and accurate P values. Please also specify the clinical covariates that were adjusted. The conclusion needs to be tone down since the authors only identified the prognostic role of the risk score, not the predictive accuracy.

**Reply 3:** We appreciate your kind suggestion. In the background section, we have added comments on the limitations of biomarkers on the efficacy prediction of ICIs in RCC patients and the prospect of genetic mutation prognostic models, which are further discussed in the introduction section. In the methods section, the clinical factors and efficacy outcomes in all the publicly available databases would be collected and described as concisely as possible in the main text and in Table 1. A total of 261 patients with advanced RCC in the CheckMate ICI therapy cohort were randomly assigned to either a training set or a validation set in a nearly 3:2 ratio through “createDataPartition” function in R package “caret” (version 6.0), which would be supplemented in the methods section in the main text. The statistical methods for assessing

the predictive accuracy have been added as you suggested. In the results section, the prognostic role of the risk score has been quantified by using HR and accurate P values as you suggested. In the conclusion section, we have lowered our tone according to your request.

**Changes in the text:**

We have modified our abstract as advised (see Page 2-3, line 40-79).

**Comment 4:** Fourth, in the introduction of the main text, the authors need to extensively review what has been known on the factors and that are associated with the treatment outcomes of ICIs, the limitations and predictive accuracy of these known predictors or predictive models, analyze the clinical needs for the new predictive model and explain why the gene-based model is potentially effective to predict the treatment outcomes.

**Reply 4:** Thank you for your valuable comment. In the introduction of the main text about what has been known on the factors associated with the treatment outcomes of ICIs, In addition to genetic characteristics such as TMB and MSI that have been described, we have added molecular characteristics, like PD-1/PD-L1 (1), as well as clinicopathological characteristics such as human endogenous lentivirus virus expression and defective antigen presentation (2), in prediction of treatment outcomes to ICIs in RCC patients. Since there are few prognostic models in RCC patients receiving ICIs, and gene mutation results in more neo-antigens, thus increasing chances for T cell recognition, and indicating better ICI (3), we might consider that signatures based on gene mutation would be potentially effective to predict the treatment outcomes.

**References:**


1. Erlmeier F, Weichert W, Schrader AJ, et al. Prognostic impact of PD-1 and its ligands in renal cell carcinoma. Med Oncol 2017;34:99.
2. Au L, Hatipoglu E, Robert de Massy M, et al. Determinants of anti-PD-1 response and resistance in clear cell renal cell carcinoma. Cancer Cell 2021;39:S1535-6108(21)00543-2.
3. Jardim DL, Goodman A, de Melo Gagliato D, et al. The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker. Cancer Cell 2021;8;39(2):154-173.


**Changes in the text:**

We have modified our “Introduction” section as advised (see Page 4, line 112-118; Page 5, line 120-122).

**Comment 5:** Fifth, the methodology of the main text needs to describe the research design, clinical factors and efficacy outcomes in the databases, and whether the sample from the databases is large enough for generating and validating the predictive model. In statistics, please explain whether the clinical covariates adjusted are adequate for the adjustment and describe the details of the multiple Cox regression analysis. The authors must be aware of the differences between the identifications of associated factors and the adjustment of clinical covariates to ascertain the independent prognostic role of the risk score. Based on the text, the authors focused on the former.

**Reply 5:** Thank you very much for the professional comments. we have supplemented the basic clinical information of the five public databases in the methodology of the main text. We are sorry for the limitation that our research is only a retrospective study based on publicly available databases, in which situation we have tried as far as possible to include all the clinical samples that are proved to receive ICIs or not, as well as all the clinical factors that are available. The mutation classifier should be validated in prospective studies with larger cohorts from multiple centers with accurate design of sample size and clinical variables in the future. the details of the multiple Cox regression analysis have been supplemented in the section “Construction of

the mutation classifier". Thank you again for your professional and pertinent suggestions on methodology and statistics.

**Changes in the text:**

We have modified our text as advised (see Page 5-6, line 145-153; Page 7, line 204-210).

**Comment 6:** Finally, please consider to cite the below related paper: Ning K, Wu Z, Zou X, Liu H, Wu Y, Xiong L, Yu C, Guo S, Han H, Zhou F, Dong P, Zhang Z. Immune checkpoint inhibitors further aggravate proteinuria in patients with metastatic renal cell carcinoma after long-term targeted therapy. Transl Androl Urol 2022;11(3):386-396. doi: 10.21037/tau-21-1015

**Reply 6:** Thank you very much for the constructive suggestions. This article inspires us to think about the limitations of our research about clinicopathological characteristics such as exacerbation in proteinuria after ICI therapy, may also influence the prognosis of RCC patients, more adverse events post ICI treatment should be considered in future study.

**Changes in the text:**

We have cited this paper in the discussion section in our manuscript (see Page 4, line 112-118; Page 19, line 607-610).

**Review comment-reviewer B**

**Comment 1:** You used lasso regression to identify genes that may be risk factors, but what was the outcome used to limit the number of genes to 10?

What outcomes did you use when you narrowed it down to 10 genes?

**Reply 1:**

Thank you for your valuable suggestion. We have discussed the outcome used to limit the number of genes to 10 in the "Methods" section with the description "10 genes and their coefficients were retained, with the penalty parameter (λ) decided by the minimum criteria" (See Page 7, Line 193-194).

**Comment 2:** Could you please explain the "survminer" model in detail?

**Reply 2:** Thank you for your valuable comment. The "survminer" R package provides functions for facilitating survival analysis and visualization, in which we used the "surv_cutpoint" function to determine the optimal cutpoint for one or multiple continuous variables at once, providing a value of a cutpoint that correspond to the most significant relation with survival.

**Review comment-reviewer C**

**Comment 1:** What are the relevant characteristics of the tumor microenvironment of RCC? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

**Reply 1:** We appreciate your kind suggestion. We have discussed the characteristics of the tumor microenvironment of RCC in single-cell level in the "Results" section (See Page 15, Line 472-482). And our mutation classifier might provide some predictive information for determining when Opdualag therapy can be applied to the treatment of RCC (See Page 19, Line 582-593).

**Comment 2:** In this study, bioinformatics approaches were employed to develop the model. It is suggested to add further functional experiments to study its role in vivo and potential molecular mechanisms.

**Reply 2:** Thank you for your valuable suggestion. We are sorry that we failed to conduct further functional experiments to study the role of our mutation classifier in vivo in this paper due to the limits of time to validate all of the 10 genes. Further investigation through in vivo and in vitro experiments would be conducted to fully elucidate the impact of the 10-gene mutation classifier.

**Comment 3:** What are the biggest advantages and disadvantages of the 10-gene mutation classifier in this study? It is recommended to add relevant contents in the discussion.

**Reply 3:** Thank you very much for the professional comments. Although TMB has controversial value in predicting the OS of ICI treatment in patients with advanced RCC, some genetic mutations have been shown to affect the function of tumor immune-related pathways and reshape the tumor immune microenvironment, thus affecting ICI response, so our prognostic model based on the mutation profile in advanced RCC is novel with some theoretical basis (See Page 17, Line 533-549). The limitations of our mutation classifier can be found in Page 19, Line 594-612.

**Comment 4:** The description of some methods in this study is too simplistic, please describe in detail.

**Reply 4:** Thank you very much for the constructive suggestions. We have indicated the sources of publicly available databases and cited the papers from which the R packages were derived as far as we could. We are looking forward to your further questions and we are pleased to provide detailed answers to your doubts about data and codes.

**Changes in the text:** We have modified our text as advised (See Page 6, Line 174).

**Comment 5:** How to determine the criteria according to this study to screen the most suitable population for ICIs treatment? It is suggested to add relevant contents.

**Reply 5:** Thanks for your kind comment. The most suitable population for ICIs treatment are those patients with higher risk scores calculated by the formula (See Page 11, Line 319-323) and stratified by the optimal cutpoint determined by the "surv_cutpoint" function in "survminer" package in R software.

**Changes in the text:** We have modified our text as advised (See Page 7, Line 200-201).

**Comment 6:** What are the cell types and expression characteristics in the immune microenvironment of RCC? How are the dynamic changes and connections between cells in different tissues? It is recommended to add relevant content.

**Reply 6:** This is a very good question. RCC are heterogeneous malignancies thought to arise from kidney tubular epithelial cells, and the effect of immune heterogeneity on clinical outcome in RCC has not been fully characterized. We have discussed some of the studies about RCC using single-cell sequencing technology in the "Results" section (See Page 15, Line 471-481), and we are planning to conduct large-scale single-cell sequencing studies in the future to further reveal the intercellular interactions in the immune microenvironment of RCC.

**Comment 7:** The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Tumor mutational burden and immune signatures interplay in renal cell carcinoma, Ann Transl Med, PMID: 32355713". It is recommended to quote this article.

**Reply 7:** We appreciate your kind suggestion. We have carefully read this paper and made supplements and references in the "Introduction" section.

**Comment 8:** What impact will the tumor microenvironment have on the immune checkpoint inhibitor response? It is recommended to add related content.

**Reply 8:** Thank you for your valuable suggestion. We have discussed the certain subgroups of immune cells and expression levels of immune checkpoints such as PD1, CTLA4, TIGIT, TIM-3 and LAG3 for eliciting the favorable response within patients with ICI-treated advanced RCC (See Page 15-16, Line 471-492).

**Review comment-reviewer D**

1. Figure 1

Please explain TMB in the legend.

Reply: We appreciate your kind suggestion. We have revised the legend of Figure 1 as required.

Changes in the text: We have modified our text as advised (see Page 26, Line 780-781).

2. Figure 2

Please explain AUC, Lasso, HR in the legend.

Reply: We appreciate your kind suggestion. We have revised the legend of Figure 2 as required.

Changes in the text: We have modified our text as advised (see Page 28, Line 792-793).

3. Figure 3

Please explain HR and CI in the legend.

Reply: We appreciate your kind suggestion. We have revised the legend of Figure 3 as required.

Changes in the text: We have modified our text as advised (see Page 29, Line 805).

4. Figure 4

Please explain HR in the legend.

Reply: We appreciate your kind suggestion. We have revised the legend of Figure 4 as required.

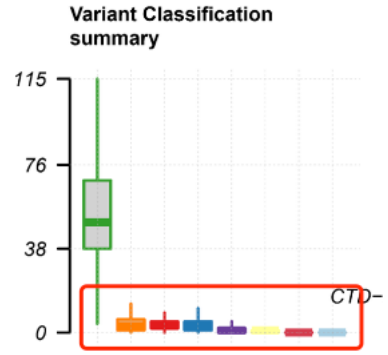
Changes in the text: We have modified our text as advised (see Page 30, Line 817).

5. Figure 5

Please explain BP, MF, CC, and FDR in the legend.
Reply: We appreciate your kind suggestion. We have revised the legend of Figure 5 as required.
Changes in the text: We have modified our text as advised (see Page 31, Line 830-831).

6. Figure 6
Please explain HR in the legend.
Reply: We appreciate your kind suggestion. We have revised the legend of Figure 6 as required.
Changes in the text: We have modified our text as advised (see Page 33, Line 844).

7. Figure S2
a) Please explain SNV, MSKCC, RCC, and ICI in the legend.
b) Please provide the description of the x-axis.




Reply: We appreciate your kind suggestion. We have revised the Figure S2 as required.
Changes in the text: We have modified our text and the Figure S2 as advised (see Page 36, Line 862-863).

8. Figure S3
Please explain MSKCC in the legend.
Reply: We appreciate your kind suggestion. We have revised the legend of Figure S3 as required.
Changes in the text: We have modified our text as advised (see Page 37, Line 865-866).

9. Figure S4
Please explain ROC, AUC, OS, MSKCC, RCC, and ICI in the legend.
Reply: We appreciate your kind suggestion. We have revised the legend of Figure S4 as required.
Changes in the text: We have modified our text as advised (see Page 38, Line 882-885).

10. Figure S5 and S6
Please explain TCGA in the legend.
Reply: We appreciate your kind suggestion. We have revised the legend of Figure S5 and Figure S6 as required.
Changes in the text: We have modified our text as advised (see Page 40, Line 899; Page 41, Line 906-907).