Peer Review File

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Reviewer A

1) First of all, my major concern regarding this study is the poor predictive accuracy of the prognosis prediction model, AUC=0.658, which is much lower than an acceptable level. The other major concern regarding the methodology is no external validation sample to validate the model. Based on this, this is a failed study. The authors need to consider whether such results deserve to be reported.

Reply 1: In order to verify the accuracy of the model, we selected GSE37745 data set for external verification, which included 66 patients with LUSC. The results showed that there was a significant difference in the survival rate between the high and low risk groups(p=0.012), and the AUC value of the area under the ROC curve was 0.644, indicating that the model had certain predictive value.

In addition, ROC analysis was performed on clinical indicators of LUSC, such as age, stage, T, N, M, etc., together with the risk score. Compared with all clinical indicators, the AUC value of risk score was the largest, indicating that the prognostic model had certain predictive value.

The analysis of the validation model and clinical indicators showed that the AUC value was not very high, but relatively stable. We believe that it has potential prognostic value for LUSC patients. Of course, it needs to be further verified in large-scale and prospective studies.

Changes in the text: We added the above analysis to the Construction and evaluation of the prognostic model in the Methods and Outcomes section (see page 5, lines 142-144; page 6, lines 189-191; page 9, lines 284-292).

2) Second, the abstract needs some revisions. The background did not indicate the knowledge gaps on the prognosis prediction model in LUSC and why the TMB and immune response-based biomarkers is potentially able to accurately predict the prognosis. The methods need to describe the generation of the training and validation samples, and clinical and pathological variables in the databases, as well as the prognosis outcome. Methods for assessing the predictive accuracy are also needed. The results need to quantify the findings by providing outcome values of different groups, as well as accurate P values. Please also report the AUC value. The conclusion is overstated as I commented above. Please have comments on the limitations of this study.

Reply 2: We have revised the abstract, which includes background, methods, results and conclusions, as well as limitations of the study.

Changes in the text: we have modified the abstract as advised (see Pages 2-3, lines 45-85).

3) Third, the introduction of the main text needs to review what has been known on prognostic factors in LUSC, whether there were prognosis prediction models in LUSC, their predictors used, and limitations and predictive accuracy of these models, and please further explain why TMB and immune response biomarkers could accurately predict the prognosis. These information is important and should be clearly clarified.

Reply 3: In the discussion section, we added the prognosis and prediction model of lung squamous cell carcinoma, and listed some related studies. Each study has its own characteristics and new findings. However, our study for the first time combined TMB with immune genes to construct an immune-related gene prognostic model based on TMB to predict the prognosis of LUSC patients, which is more comprehensive and persuasive, and can better reflect TMB and immune characteristics. See the revision section for more information.

Changes in the text: we have modified our text as advised, the detailed information is in the discussion section of the article. (see Pages 10-11, lines 331-349, 359-365).

4) Fourth, in the methodology of the main text, please describe the research design, the generation of training and validation samples, the clinical and prognosis outcome variables in the databases, and threshold AUC values for a good predictive model. Please ensure $P<0.05$ is two-sided.

Reply 4: The research design has been added to the methods section. Other problems are also described in the methodology.

Changes in the text: we have modified our text as advised, the detailed information is in the methodology section of the article. (See Page 5-6, lines 138-145, lines 163-165, lines 189-196).

Reviewer B

In the present study, the TMB was found to be associated with clinicopathological characteristics and survival outcomes, and the high TMB group showed a better survival rate. Moreover, the authors identified five TMB-related hub immune genes

(TINAGL1, FGFR2, CTSE, SFTPA1 and IGHV7-81) and constructed a prognostic model based on these genes. The model showed good performance in predicting the survival risk of LUSC patients, indicating its potential clinical value in guiding personalized treatment and follow-up for LUSC patients. The study demonstrated the potential of TMB and immune-related genes as biomarkers for predicting the prognosis of LUSC. These findings may provide new insights into the underlying mechanisms of LUSC progression and open new avenues for developing novel therapeutic strategies. However, further prospective studies are needed to validate the findings and to investigate the potential applications of TMB and immune-related genes in clinical practice.

The manuscript needs further editing to meet the requirements for publication. The specific issues are as follows:

1) In the Abstract, the authors should introduce the background and current situation of the research field, and why the research in this field is important and then comes the purpose of the study.

Reply1: We have revised the abstract (see page 2, lines 46-49).

2) Considering the importance of tumor mutation burden in this study, the authors should elaborate in detail on the research progress of tumor mutation burden in lung cancer and the significance of this study.

Reply2: We have elaborated in the introduction and discussion section. (See page 4, lines 115-123; page 11, lines 349-357).

3) Kaplan-Meier analysis, ROC curve, risk curve, and nomogram need to be detailed explained on how to analyze.

Reply3: We have explained these in detail in the methods section. (See page 5, line 166; page 6, lines 196-198).

4) In the Landscape of mutation profiles in LUSC of the results, the description of "The somatic mutation data of LUSC was downloaded from TCGA database. Varscan software was used to analyze the mutation data (MAF file), and "maftools" software package was applied to draw the mutation graph. The tcgaComapare function in maftools was used to calculate the TMB of LUSC, and compare it with the other cohorts of TCGA." should not be given a long description cause of the results need to focus on the description of the results. The description of all methods below needs to be simplified in Results.

Reply4: We have modified it, as well as all methods for the other parts covered in the results. (See page 7, lines 213-214, lines 231-232; page 8, lines 242-243, line 260)

5) The results in the Results need to be described in detail rather than simply what work has been done, such as the results in Fig. 1A and Fig.1D.
Reply5: We have described the results. (See page 5, lines 142-144; page 6, lines 189-191; page 9, lines 284-292).

6) Many conclusions in Results are inaccurate, such as KEGG analysis indicated that DEGs markedly enriched in Th1 and Th2 cell differentiation and cytokine cytokine receptor interaction (Figure 3D). In addition, the GO analysis should be clarified in BP, CC and MF.
Reply6: We have modified it and clarified the GO analysis. (See page 8, lines 245-256).

Reviewer C

In this study, the authors constructed and validated a model based on the tumor mutational burden for predicting the prognosis of patients with lung squamous cell carcinoma. This study is interesting and can extend our knowledge in this field. The methods used in this article are generally correct, and some modifications are needed.
1) Lines 195 to 199: these sentences should be moved to the methods section.
Reply1: These sentences were removed and described in the method.

2) Lines 215 to 217, line 151: the optimal threshold was 2.61. Please report the methods used to determine the optimal threshold in the methods section.
Reply2: We have explained in the method, The surv_cutpoint function was used to obtain the optimal cut-off value. (See page 5, lines 146-161; page 7, lines 213).

3) Figure 6B: the roc curve should be removed. ROC curve cannot be used to analyze time-to-event data because the time of follow-up is a confounding factor. In addition, figures 8a and 8b should be removed.
Reply3: The figures have been removed and used as supplementary figures.

4) Figures 9a and 9b, because T, M and N have been included in stage, these three parameters cannot be analyzed with stage. You can analyze T, M and N separately or only analyze the stage.
Reply4: After deleting the stage, we reanalyzed the data and modified it in the article. (See Figures 9a and 9b).

5) A previous study has investigated the TMB-associated model in LUSC. Please discuss the strength of this study in the discussion section.

Reply5: We add some related studies and describe the advantages of our study in the discussion section. (See page 11, lines 342-357).

6) Line 147, please report the detail of the score calculation.

Reply6: We have reported in detail. (See page 5, lines 159-161).

7) Line 218, Kaplan Meier should be revised as Kaplan-Meier.

Reply7: We have modified it.

8) The authors performed GO and KEGG enrichment analyses, but the clinical implications of these analyses were not discussed in the discussion section.

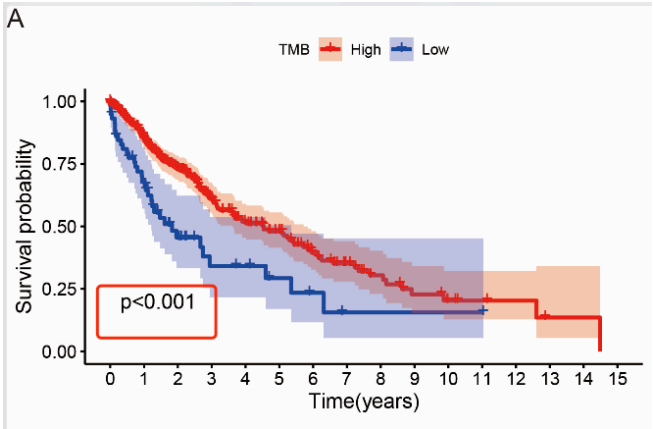
Reply8: We have explained this in the discussion section. (See page 11-12, lines 369-377).

Reviewer D

1. Figure 3

In figure, p<0.001 which is different from the legend, please check and revise.

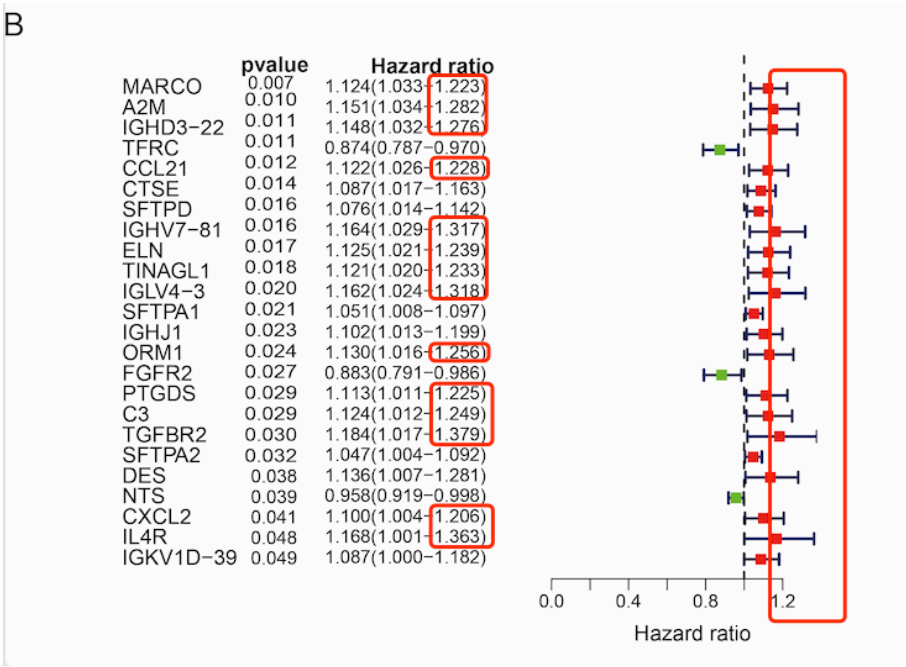
characteristics. (A) Lower TMB levels are related to the poor prognosis of LUSC patients, P=0.035. (B) The TMB level was related to stages, P=0.012. TMB, tumor

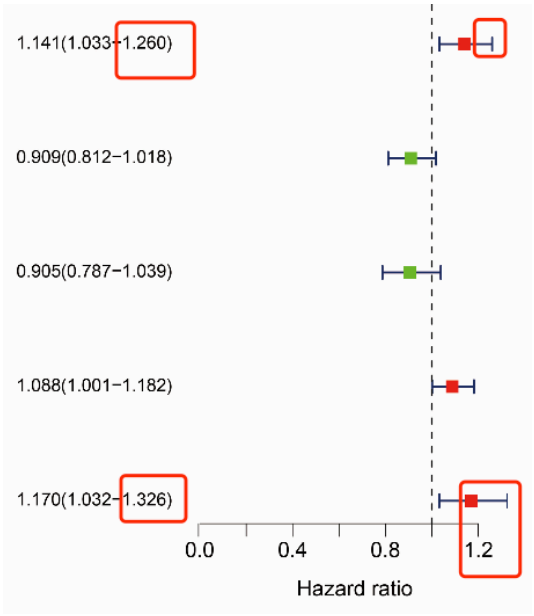


Reply: Thank you for your reminder. We have revised it

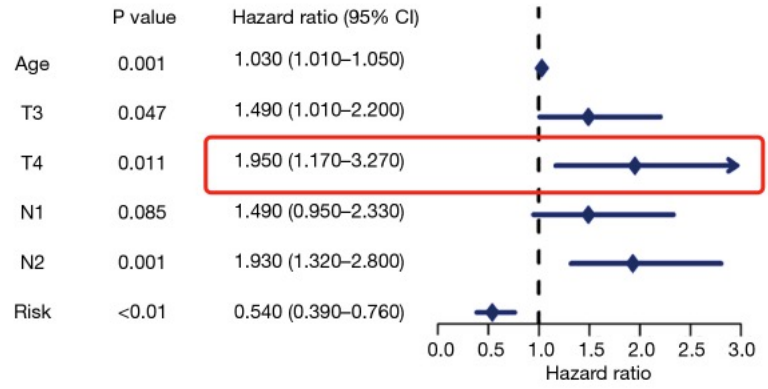
2. Figure 5

To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows.





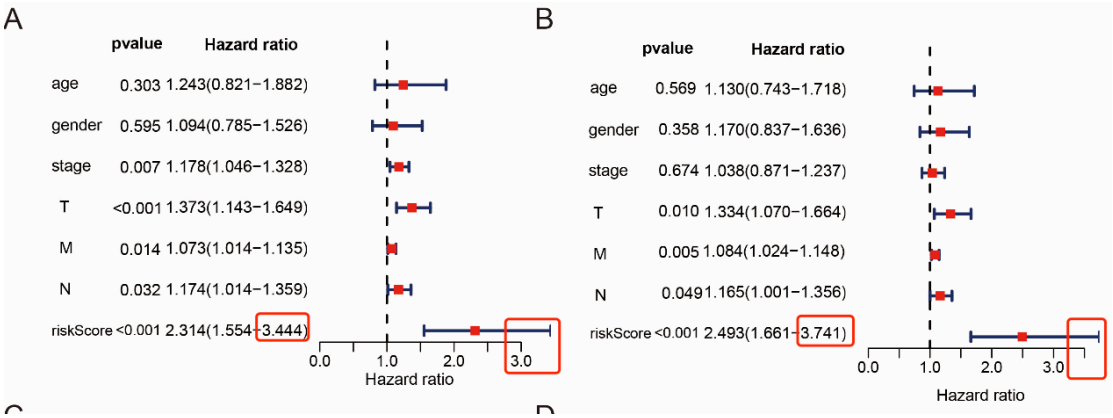
Here's an example for your reference:



Reply: Thank you for your reminder. We have revised it

3. Figure 9

To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows.



Reply: Thank you for your reminder. We have revised it