Peer Review File

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Reviewer A

This bioinformatics study constructed a mast cell-related prognostic model for NSCLC using TCGA and GEO data by Univariate Cox and LASSO regression. The high-risk group was associated with low resting mast cell infiltration and ICI expression, and relatively low drug sensitivity. I have several questions/comments.

Comment 1. In the "Introduction" section, the authors mentioned "early mast cell infiltration in various human and animal tumors". Could you please explain which function dose mast cell plays, anti-tumor or pro-tumor? According to my intuition, mast cells as part of the immune system may play an anti-tumor role in the TME.

Reply 1: The role of mast cells in tumors is multifaceted, including promoting tumor growth, angiogenesis, and immune escape. First, mast cells can secrete a variety of growth factors and cytokines, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), etc., which can promote tumor growth and metastasis. Second, mast cells can also promote tumor angiogenesis. Tumors need to obtain nutrients and oxygen through new blood vessels, and mast cells can secrete factors such as VEGF to stimulate angiogenesis. Finally, mast cells can also contribute to tumor immune escape. Mast cells can secrete factors such as IL-10 to inhibit the activity of immune cells such as T cells, so that tumors can escape the attack of the immune system. Therefore, mast cells play an important role in the occurrence and development of tumors, and are one of the important targets for research and treatment of tumors.

Changes in the text: Page 3/line 88-100.

Comment 2. Could you please perform a validation of your risk model using other external independent datasets?

Reply 2: Dear reviewer, I appreciate your review of our research. It is mentioned in your review that we need to use an external independent dataset to validate the results of our analysis. I regret to inform you that we were unable to validate our analysis results using external independent datasets as we were unable to obtain data of sufficient quantity and diversity. We fully understand the importance of validating our analysis results, so we have done our best to validate our analysis results on internal data, and fully demonstrate this in the article. We also encourage other researchers to use our method in future studies and to validate it on more data. We hope you will understand our predicament and make an overall assessment based on your assessment of other aspects of our research. If you still have any questions or suggestions, please feel free to contact us. Thank you again for your interest in and review of our research.

Comment 3. Could you please make a brief explanation about the results of the GSEA pathway enrichment analysis? It is confusing for readers to understand the relationship between the pathway and the prognosis.

Reply 3: The differential enrichment of these pathways may also provide clues for further research to better understand biological differences between different patient populations. In addition, these findings can help optimize individualized treatment regimens and are expected to ultimately improve patient survival and quality of life.

Changes in the text: Page 7/line 212-213.

Comment 4. Based on your results, T cells CD4 memory resting were also enriched in the low-risk group and negatively associated with risk score just as mast cells resting, which may be a co-factor in the risk model and influence the prognosis. It is better to discuss the interaction between them.

Reply 4: Several studies have shown that CD4 memory T cells can stimulate the activation of mast cells and release cytokines, thereby regulating immune and inflammatory responses. In addition, resting mast cells can also affect the activation and expansion of CD4 memory T cells, thereby affecting the strength and direction of the immune response. These findings provide an important reference for further understanding the regulatory mechanism of the immune system and the occurrence and development of related diseases.

Changes in the text: Page 9/line 279-285.

Reviewer B

Dr. Yang and their colleagues constructed a mast cell risk model for NSCLC based on TCGA and GEO. Based on that, they explored the relationships between the risk-scoring model, immune infiltration cells, TMB, and immune checkpoint inhibitors.

Comment 1. Due to the complexity of bioinformatic analysis, we cannot verify the accuracy of the study.

Reply 1: We fully understand that the complexity of bioinformatics analysis can present difficulties in verifying the accuracy of studies. However, various approaches have been taken to minimize analytical errors and increase the reliability of the results. For example, we used standardized processes and software during data preprocessing and analysis, performed multiple validations on the results, and compared the analysis results with existing literature. Although we cannot guarantee that our analysis is completely error-free, we believe that our results are reliable and can provide valuable information for subsequent research and treatment.

Comment 2. The two survival lines in Fig 2A overlap. So the results should be interpreted with caution.

Reply 2: Thank you very much for your reminder. In this study, we analyzed the overall survival rate of non-small cell lung cancer. The early high-risk group indicates poor prognosis. For the repeated part, there is no difference in the late stage of non-small cell lung cancer.

Changes in the text: Page 6/line 200.

Comment 3. I suggest that previous studies should be fully discussed and compared and connected with this study in the Discussion section instead of simply repeating the results.
Reply 3: We have modified in the text.
Changes in the text: Page 9/line 298-311.

Comment 4. Please discuss the limitations of this study.
Reply 4: However, the study also had some limitations. First, this is a database analysis that requires further in vitro experimental studies to validate. Second, the sample size used in this study was small, and further expansion of the study scale is required. In addition, the data used in this study are horizontal research data, and further longitudinal research is needed to verify the stability and reliability of its results.
Changes in the text: Page 9/line 298-302.

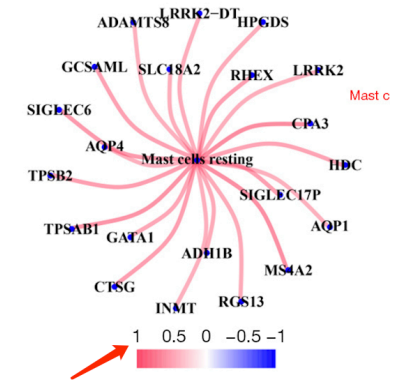
Reviewer C

Comment 1: Figure 1
a) Please explain FC, GO.
Reply: Log FC: log fold change. Gene Ontology: GO.
Changes in the text: page 13/line 583.

b) Please provide a clearer version of figure 1A, the current version cannot be seen clearly.
Reply: We have recently revised in Figure 1A.

c) Please provide the explanation of the color bar.

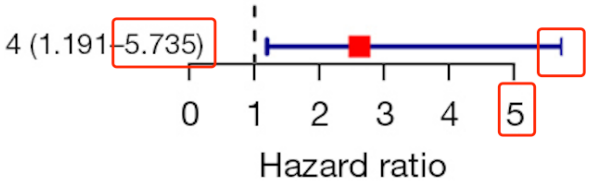
D E



Reply: Color scale bar represents correlation.
Changes in the text: page 13/line 585.

Comment 2: Figure 2
a) To standardize the results, the part that exceeds the horizontal coordinates should be indicated

by arrows.



Reply: We have recently revised in Figure 2B

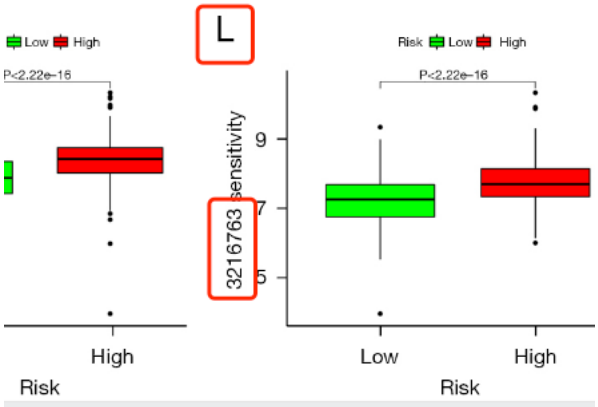
b) Please provide the meaning of the symbol "*, ***" in the legend.
Reply: *, *represents < 0.05; *** represents < 0.001.*
Changes in the text: page 14/line 596.

Comment 3: Figure 4
a) Please explain LUAD in the legend.
Reply: LUAD, lung adenocarcinoma.
Changes in the text: page 15/line 606.

b) Please explain the meaning of * in the legend.
Reply: *, *represents < 0.05.*
Changes in the text: page 15/line 608.

Comment 4: Figure 6
Please check if the main text matches the figure.

256 6K), and SB216763 (Figure 6L). So, accurate prediction can potentially help to



Reply: We have recently revised in Figure 6L.

Comment 5: References/Citations
a) There are 2 reference lists in the file, please keep the correct one and delete another one.
b) Reference 17-19 are not cited in the main text, please indicate where to cite.
Reply: We have recently revised all references.