Peer Review File

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**For the reviewer A:**

**Comment1:** The Authors submitted a manuscript on the prognostic relevance of alternative splicing events in LUAD.

I thank the Authors for the work they've done. However, my opinion is that their unsupervised analyses lack a solid biological background and a clear hypothesis. They are therefore not informative for either cancer cell biology or clinical phenotypes in LUAD.

**Reply:** Thank you for your comments. We have realized the problem and tried to modify the article.

**Changes in the text:** We have changes in every place that we thought were wrong.

**For the reviewer B:**

**Comment1:** 1) How to construct a prognostic signature based on AS? What is the role in the tumor immune microenvironment in LUAD? Suggest adding relevant content.

**Reply:** Thank you for your comments. We have realized the problem and added relevant content.

**Changes in the text:** We have modified our text as advised (see page 2,line 41-42、page 4,line 114-117)

**Comment2:** 2) What is the correlation between AS and immune cell infiltration events and patient prognosis? Suggest adding relevant content.

**Reply:** Thank you for your comments. We have realized the problem and added relevant content.

**Changes in the text:** We have modified our text as advised (see page 4,line 117-119).

**Comment3:** 3) Suggest integrating the information of LUAD patients from TCGA and evaluating the AS profiles from the perspectives of gender and subtypes.

**Reply:** Thank you for your comments. This article is more inclined to focus on the impact of variable splicing on the prognosis of lung adenocarcinoma patients, so the relationship between AS and gender and subtype is not considered for the time being. We believe that in Figure 2E, the model of lung adenocarcinoma patients has been evaluated based on clinical factors such as gender, age and stage, and the results suggest that gender has no absolute effect on the prognosis of LUAD, but clinical stage does, which is consistent with the reality. However, in Figure 4, we further analyzed the correlation between AS and patients with different stages.

**Changes in the text:** Sorry, the article has not been changed for this.

**Comment4:** 4) What is the greatest advantage of the prediction model in this study? What is the biggest problem we are facing? It is suggested to add relevant content to the discussion.

**Reply:** Thank you for your comments. We have realized the problem

and added relevant content.

**Changes in the text:** We have modified our text as advised (see page 9,line 279-284).

**Comment5:** 5) Figure 1 is not clear enough. It is recommended to provide clearer figure again.

**Reply:** Thank you for your comments. We have realized the problem and changed it.

**Changes in the text:** We have changed Figure 1.

**Comment6:** 6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "A comprehensive characterization of alternative splicing events related to prognosis and the tumor microenvironment in lung adenocarcinoma, Ann Transl Med, PMID: 35571443". It is recommended to quote this article.

**Reply:** Thank you for your comments. We have realized the problem and added relevant content.

**Changes in the text:** We have modified our text as advised. (see page 4,line 109-116).

**Comment7:** 7) This study is based on bioinformatics analysis. It is recommended to increase in vivo experimental studies, which may be more meaningful.

**Reply:** Thank you for your comments. In the later stage, we will consider selecting several key genes to supplement in vivo experiments.

Changes in the text: Sorry, the article has not been changed for this.

**For the reviewer C:**

**Comment1:** 1) First, the title needs to indicate the development of the prognosis prediction model based on As and other clinical variables. My major concern for this study is the poor predictive accuracy of the prediction model in particular the prediction of 3- and 5-year prognosis. I suggest the authors to change the focus of this study to be the prognostic role of As, not to develop the nomogram.

**Reply:** Thank you for your comments. We have realized the problem and modified the title.

**Changes in the text:** We have modified our text as advised (see page 1,line 3-4)

**Comment2:** 2) Second, the abstract is not adequate. The background need to explain why As is a potentially important prognostic factor in LUAD. The methods need to describe the clinical factors and prognosis outcomes in the datasets and how the independent prognostic role of As was ascertained. The results need to briefly describe the clinical sample in the dataset and the HR and accurate P value for the prognostic role of the ASE score. The conclusion needs comments for the clinical implications of the findings.

**Reply:** Thank you for your comments. We have realized the problem and modified the essay.

**Changes in the text:** We have modified our text as advised (see page 1-2,line 34-35、page 2,line 55-56)

**Comment3:** 3) Third, the introduction of the main text needs to briefly review what has been known on the prognostic roles in LUAD, their limitations, and why there is a need to examine new prognostic biomarkers. Please also explain why As is potentially associated with prognosis in LUAD and what the potential clinical significance of this research focus is.

**Reply:** Thank you for your comments. We have realized the problem and modified the essay.

**Changes in the text:** We have modified our text as advised (see page 3,line 93-96、line99-101).

**Comment4:** 4) Fourth, in the methodology, the authors need to describe the clinical sample, clinical factors, and prognosis outcomes in the dataset. In statistics, please describe the test of the independent prognostic role of As-based score and the adjusted clinical covariates.

**Reply:** Thank you for your comments. We have realized the problem and modified the essay.

**Changes in the text:** We have modified our text as advised (see page 4,line 131-132、page 5 140-144).

**Comment5:** 5) Finally, please cite several related papers: 1. Lin X, Zhou T, Hu S, Yang L, Yang Z, Pang H, Zhou X, Zhong R, Fang X, Yu Z, Hu K. Prognostic significance of pyroptosis-related factors in lung adenocarcinoma. J Thorac Dis 2022;14(3):654-667. doi: 10.21037/jtd-22-86. 2. Dora D, Vörös I, Varga ZV, Takacs P, Teglasi V, Moldvay J, Lohinai Z. BRAF RNA is prognostic and widely expressed in lung adenocarcinoma. Transl Lung Cancer Res 2023;12(1):27-41. doi: 10.21037/tlcr-22-449. 3. Nakajima N, Yoshizawa A, Rokutan-Kurata M, Noguchi M, Teramoto Y, Sumiyoshi S, Kondo K, Sonobe M, Hamaji M, Menju T, Date H, Haga H. Prognostic significance of cribriform adenocarcinoma of the lung: validation analysis of 1,057 Japanese patients with resected lung adenocarcinoma and a review of the literature. Transl Lung Cancer Res 2021;10(1):117-127. doi: 10.21037/tlcr-20-612.

**Reply:** Thank you for your comments. We have realized the problem and added relevant content.

**Changes in the text:** We have modified our text as advised. (see page 10,line 314、322、324).