Peer Review File

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Review Comments-Reviewer A

1) First of all, my major concern regarding this study is the questionable prognosis predictive performance of the seven gene prognosis signature model because of the AUC values lower than 0.7, suggesting this is a failed study of the development and validation of the prognosis prediction model. The current data can only assess the prognostic role of the even gene prognosis signature, not the predictive accuracy. The paper must be substantially revised.

Response: Thanks a lot for your good suggestion. The current prognosis prediction model was developed using data from public databases. We also conducted follow-up analyses as a way to validate the results in clinical cohorts.

Changes in the text: None.

2) Second, the abstract needs to indicate the clinical significance and limitations of known prognostic and prognosis predictive models in the introduction, the methods need to describe the generation of training and validation samples and statistical methods for assessing the predictive accuracy, the results need to report HR and accurate P values to indicate the prognostic role, and the conclusion needs comments for the clinical implications of the findings.

Response: Thanks a lot for your good suggestion. The clinical significance was indicated in the abstract (Conclusions in the Abstract). We have added the limitations of known prognostic and prognosis predictive models and revised our manuscript (Introduction paragraph 3). We have added the generation of training and validation samples and statistical methods in revised our manuscript (Methods paragraph 1 and paragraph 6). We have added HR and P values in revised our manuscript (Results paragraph 4). We have added the clinical implications of the findings (Discussion paragraph 6).

Changes in the text: we have modified our text as advised (see Page 3, line 82-88; Page 3, line 98-108 and Page 5, line 151-157; Page 10, line 328 and Page 11, line 329-335).

3) Third, the introduction of the main text needs to have a detailed review on the prognostic biomarkers and prognosis prediction models in lung cancer, have comments on the limitations of prior studies including the predictive accuracy, and explain why M1 macrophage-related gene based predictive models are potentially accurate for predicting the prognosis. Please also clarify the clinical significance of this research focus.

Response: Thank you very much for your good suggestion. We have reviewed on the prognostic biomarkers and prognosis prediction models in lung cancer. We have explained why M1

macrophage-related gene based predictive models for predicting the prognosis. We have added the information and revised our manuscript (Introduction paragraph 3).
Changes in the text: we have modified our text as advised (see Page 3, line 82-88).

4) Fourth, in the methodology of the main text, please describe the research design, generation of training and validation samples, and the clinical factors and prognosis outcomes in the databases. The authors need a separated paragraph to describe the statistical methods for assessing the prognostic role and the diagnostic accuracy.

Response: Thanks a lot for your good suggestion. We have added the information and revised our manuscript (Methods paragraph 1 and Methods paragraph 6).
Changes in the text: we have modified our text as advised (see Page 3, line 98-108 and Page 5, line 151-157).

5) Finally, please consider to cite the below related papers: 1. Li H, Ge Y, Fei G, Wang Z, Wang S, Wei P. Development and validation of a combined ferroptosis and immune prognostic signature for lung adenocarcinoma. Transl Cancer Res 2022;11(10):3620-3633. doi: 10.21037/tcr-22-992. 2. Gong Z, Li Q, Li J, Xie J, Wang W. A novel signature based on autophagy-related lncRNA for prognostic prediction and candidate drugs for lung adenocarcinoma. Transl Cancer Res 2022;11(1):14-28. doi: 10.21037/tcr-21-1554. 3. Peng L, Ji J, Zhang C, Wu Z, Sun Y, Fan K, Du W, Liu A, Jiao W. Development and validation of a prognostic risk signature for lung adenocarcinoma constructed by six ferroptosis, necroptosis, and pyroptosis-related lncRNAs. J Thorac Dis 2022;14(10):3955-3974. doi: 10.21037/jtd-22-1151.

Response: Thanks a lot for your good suggestion. Indeed, as you mentioned that there were some previous articles in lung cancer. We have cited the related papers in our manuscript (Discussion paragraph 5).
Changes in the text: we have modified our text as advised (see Page 10, line 312-321).

Review Comments-Reviewer B

1. Lines 56 to 58, which had better~~~~. This sentence should be removed.

Response: Thanks a lot for your good suggestion. This sentence has been removed.
Changes in the text: we have modified our text as advised (see Page 2, line 59).

2. Line 82, however, the role of macrophages~~~~. It should be revised as: however, the role of macrophages-related genes.

Response: Thanks a lot for your good suggestion. This sentence has been revised.
Changes in the text: we have modified our text as advised (see Page 3, line 84).

3. Lines 67 to 68: this sentence should be rephrased.

Response: Thanks a lot for your good suggestion. This sentence has been rephrased.

Changes in the text: we have modified our text as advised (see Page 3, line 68-69).

4. Figure 1A, what does the title of x-axis mean? What does macrophages M1 mean?

Response: Thanks a lot for your good suggestion. The title of x-axis and macrophages M1 mean the expressions of M1 macrophages-related genes. We have added the information in the Figure 1 legend.

Changes in the text: we have modified our text as advised (see Page 14, line 424-425).

5. Lines 213 to 214, Additionally, this seven-gene signature could be used to predict a total of 442 LC patient prognosis in patients from the GEO cohort. This sentence is confusing. How to predict the prognosis of 442 patients?

Response: Thanks a lot. We are sorry about this confusing sentence. We have revised this sentence.

Changes in the text: we have modified our text as advised (see Page 4, line 101-103).

6. Lines 283 to 286, the findings of this work is consistent with previous studies. Please discussed the added value of this work. In other words, did this work extend our knowledge in this field.

Response: Thanks a lot for your good suggestion. The value of this work was added in this paragraph.

Changes in the text: we have modified our text as advised (see Page 9, line 288-289).

7. Lines 296 to 307, this paragraph introduced some genes studies in this work. A sentence is needed to summarize this paragraph.

Response: Thanks a lot for your good suggestion. We have added the information in this paragraph.

Changes in the text: we have modified our text as advised (see Page 10, line 299).

8. Lines 308 to 313, three previous works have been introduced. What are the novelty of this work?

Response: Thanks a lot for your good suggestion. Recently, the prognostic gene signatures of autophagy, ferroptosis and immune were established in lung adenocarcinoma in three previous works, however, the role of macrophages-related genes in the prognosis of LC remains unclear. A systematic study was performed to identify M1 macrophage-related genes in LC using the

TCGA database and to investigate the underlying molecular mechanisms of these genes in LC patients in our study. This is the novelty of our work.
Changes in the text: we have modified our text as advised (see Page 10, line 312-321).

9. Line 124, single factor should be revised as univariate. This is the same to the line 199.
Response: Thanks a lot for your good suggestion. We have revised the information.
Changes in the text: we have modified our text as advised (see Page 7, line 199).

Review Comments-Reviewer C

This is an exploration of the markers of M1 macrophages in lung cancer based on public data. The approach is clear, and the author ultimately selected 7 relevant genes to construct their model. However, there have been previous reports on the exploration of M1 macrophages in lung cancer using public data (such as doi: 10.1016/j.heliyon.2023.e12798 and 10.21037/tlcr-22-866). The author should explain the novelty of this article, and further experimentation is needed to validate these findings.

In addition, please answer the following questions:

Q1: This article is only based on mining public databases and has no experimental validation. Therefore, I suggest that the author must add experimental or real-world sample studies.
Response: Thanks a lot for your good suggestion. We are preparing to document phenotypic experiments and mechanism studies together, and are currently collecting samples.
Changes in the text: None.

Q2: The introduction section should briefly introduce the background, motivation, and purpose of the study. There is too much statement about the content of the article, which overlaps with the methods and results in the following text. The layout needs to be streamlined.
Response: Thanks a lot for your good suggestion. We have revised the introduction.
Changes in the text: we have modified our text as advised (see Page 3, line 89-93).

Q3: In the method section (line103-112), for the selection of TCGA and GEO datasets, are these patients all non-small cell lung cancer or does it also include small cell lung cancer? For the GEO dataset, I suggest writing out the dataset sequence; for patient selection, the author needs to clarify the selection criteria for inclusion and exclusion.
Response: Thanks a lot for your good suggestion. These patients are non-small cell lung cancer. For the GEO dataset, we used the dataset GSE68465. For patient selection, we have added the selection criteria for inclusion and exclusion. We have revised the information in the method section.

Changes in the text: we have modified our text as advised (see Page 4, line 103-108).

Q4: In the method section (line115-120), the author did not mention the number and source of M1 macrophage-related genes, which refers to the 87 genes mentioned later. If so, please tell the source of this gene set. Also, why choose the top 10 genes instead of other numbers of genes, as different numbers of genes often bring different screening gene effects. Also, is the statistical significance of GO and KEGG analysis using q<0.05? If so, please clarify.

Response: Thanks a lot for your good suggestion. We have added the information of 87 M1 macrophage-related genes in the method section.

Please allow us to explain the top 10 genes. Instead of selecting only the top10 gene for subsequent analysis, we selected all 87 genes for subsequent analysis. Due to the limited space displayed in Figure 1, only top10 is displayed. The statistical significance of GO and KEGG analysis used the $q < 0.05$.

Changes in the text: we have modified our text as advised (see Page 4, line 113-114 and line 117-118).

Q5: In the method section (line123-136), "single-factor Cox analysis" should be “univariate Cox regression analysis”.

Response: Thanks a lot for your good suggestion. We have revised the information.

Changes in the text: we have modified our text as advised (see Page 4, line 123).

Q6: In the method section (line138-144), why only select 8 immune checkpoint-related genes? Can other immune checkpoints be included in the analysis, such as TNFRSF, LAG3, CD40, CD40LG, and so on?

Response: Thanks a lot for your good suggestion. These 8 immune checkpoint-related genes are commonly used.

Changes in the text: None.

Q7: In the results section (line160-170), I would like to ask if this M1 macrophage is calculated based on the CIBERSORT in the method section, or other methods. This needs to be clarified. Also, why use the top 30 genes for correlation analysis? Why not use other quantities?

Response: Thanks a lot for your good suggestion. This M1 macrophage is calculated based on the CIBERSORT in the method section. Instead of selecting only the top 30 gene for subsequent analysis, we selected all 87 genes for correlation analysis and top 30 is selected to display in Figure 1C.

Changes in the text: we have modified our text as advised (see Page 4, line 113).

Q8: In the results section (line196-217), the author constructed a model. I want to know some clinical features between the high and low-risk groups, such as stage, and the expression of the

7 model genes between the high and low-risk groups. Is the ROC curve in the GEO dataset the same as in TCGA?

Response: Thanks a lot for your good suggestion. Some clinical features between the high and low-risk groups have been displayed in the Figure 4 and supplement table S4. The ROC curve in the GEO dataset was the same as in TCGA.

Changes in the text: None.

Q9: In the results section (line 219-235), it is recommended that the author combine the risk score and clinical indicators to draw a line graph to predict the survival of patients.

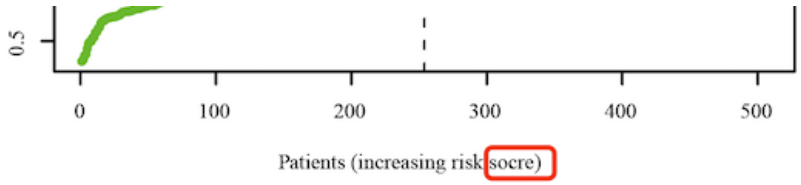
Response: Thanks a lot for your good suggestion. The two pieces of information are independent, and when combined, it may be difficult to see the effects of individual factors.

Changes in the text: None.

Review Comments-Reviewer D

1. Figure 3:

a. Please correct this typo.



b. Please revise it to "1 year".

Response: Thanks a lot for your good suggestion. We have revised Figure 3 in our manuscript.

2. Figure 4: It's better to extend the scale, please check and revise.

Response: Thanks a lot for your good suggestion. We have revised Figure 4 in our manuscript.

3. Table S2: Please check this row in the table, it seems there's error.

Table S2_2 KEGG pathway of M1 macrophage-related genes in lung cancer.

Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
activation	12/52	375/18723	3.51E-10	2.55E-07	1.76E-07	NA/NA/NA/NA/NA/NA/NA/NA/NA/NA/NA/NA	12
myeloid le	10/52	223/18723	4.80E-10	2.55E-07	1.76E-07	NA/NA/NA/NA/NA/NA/NA/NA/NA/NA	10
immune res	12/52	468/18723	4.31E-09	1.53E-06	1.05E-06	NA/NA/NA/NA/NA/NA/NA/NA/NA/NA/NA/NA	12
macrophage	7/52	106/18723	1.66E-08	3.66E-06	2.52E-06	NA/NA/NA/NA/NA/NA/NA	7
synapse pr	4/52	11/18723	1.72E-08	3.66E-06	2.52E-06	NA/NA/NA/NA	4
interleuki	7/52	128/18723	6.13E-08	9.32E-06	6.41E-06	NA/NA/NA/NA/NA/NA/NA	7
regulation	7/52	128/18723	6.13E-08	9.32E-06	6.41E-06	NA/NA/NA/NA/NA/NA/NA	7
myeloid le	8/52	220/18723	1.54E-07	1.84E-05	1.26E-03	NA/NA/NA/NA/NA/NA/NA/NA	8
phagocytos	9/52	308/18723	1.55E-07	1.84E-05	1.26E-03	NA/NA/NA/NA/NA/NA/NA/NA/NA	9
microglial	5/52	47/18723	1.91E-07	2.03E-05	1.39E-03	NA/NA/NA/NA/NA	5
cell junct	4/52	21/18723	3.06E-07	2.96E-05	2.03E-03	NA/NA/NA/NA	4
cellular d	5/52	54/18723	3.87E-07	3.43E-05	2.36E-03	NA/NA/NA/NA/NA	5
positive r	10/52	467/18723	5.24E-07	4.15E-05	2.86E-03	NA/NA/NA/NA/NA/NA/NA/NA/NA/NA	10
interleuki	6/52	110/18723	5.86E-07	4.15E-05	2.86E-03	NA/NA/NA/NA/NA/NA	6
regulation	6/52	110/18723	5.86E-07	4.15E-05	2.86E-03	NA/NA/NA/NA/NA/NA	6
positive r	9/52	427/18723	2.37E-06	0.0001574	0.0001083	NA/NA/NA/NA/NA/NA/NA/NA/NA	9
leukocyte	7/52	230/18723	3.22E-06	0.0002016	0.0001386	NA/NA/NA/NA/NA/NA/NA	7
amyloid-be	4/52	38/18723	3.64E-06	0.0002152	0.000148	NA/NA/NA/NA	4

Response: Thanks a lot for your good suggestion. We have revised Table S2 in our manuscript.