Peer Review File

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Comment 1. Any data in this manuscript did not support the title of "to improve" and "therapy response". The scope of title should be reduced to indicate the predictive value of this biomarker.

Reply: Thank you very much for your valuable comments. Here, we apologize for the discrepancy between the content of the manuscript and the title. As suggested by the reviewer, we have revised the title of the manuscript. (see Page 1, line 1-2) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 1, line 1-2

Comment 2. The number of TCGA HCC data was 371 (p.6, line 130) and that of HCC samples with immune score was 373 (Fig. 1). How the latter could be bigger than the former? In addition, the sum of high (N=182) and low (N=183)-risk group is 365 (p.12, line 243). Explain the discrepancy.

Reply: Thank you very much for your valuable comments. Here, we apologize for failing to explain the data processing process in detail.

In this study, we obtained the immune scores of 373 HCC samples from the ESTAMATE database. (see Page 6, line 131) Then, we downloaded the transcriptome data of HCC (374 cancer tissue samples and 50 adjacent normal tissue samples) and the corresponding clinical information (clinical data of 377 samples) from the TCGA database. (see Page 6, line 126) For the rationality of the study design, we included samples from the TCGA database that contain both immune score and survival time into the study.

Based on the clinical data of 377 HCC samples, we further removed these 6 samples (marked in yellow) from 373 HCC samples with immune scores, including 5 samples with a survival time of 0 days and 1 sample without survival time.

In addition, the four samples with immune score labeled blue (TCGA-DD-AACA-01 and TCGA-DD-AACA-02) and green (TCGA-ZS-A9CF-01 and TCGA-ZS-A9CF-02) as shown in the table below, we guess they may come from different locations in the same patient's

cancer tissue, respectively. Moreover, based on the clinical data of 377 HCC samples, we can only determine the survival time of the samples named TCGA-DD-AACA and TCGA-ZS-A9CF. Therefore, we selected TCGA-DD-AACA-01 and TCGA-ZS-A9CF-01 from these four samples to be included in the follow-up study. Finally, a total of 365 samples containing both immune score and survival time were used for our entire analysis.

Additionally, because of the limitations about bioinformatics tools when analyzed large amounts of data, such as normalization, data filtering, and processing missing values, this may lead to unreliable results. In a future study, we will collect more experimental and clinical data to investigate the association between the expression levels of ferroptosis-related genes and the development of HCC, especially the three ferroptosis-related genes used for the construction of the prognostic model. Relying solely on bioinformatics is a drawback of this study. In the revised manuscript, we have added a description of the defect that this study failed to be verified via experimental studies. (see Page 18, line 382) Thank you again for your constructive comments.

Changes in the text: see Page 6, line 126; see Page 6, line 131; see Page 18, line 382

373 samples with immune score	377 samples with clinical data
TCGA-2V-A95S-01（Missing）	
TCGA-BW-A5NP-01（0 Day）	
TCGA-BW-A5NQ-01（0 Day）	
TCGA-CC-A5FU-01（0 Day）	
TCGA-CC-A5FV-01（0 Day）	
TCGA-DD-AACA-01	TCGA-DD-AACA（2301 Day）
TCGA-DD-AACA-02	
TCGA-RC-A6M3-01（0 Day）	
TCGA-ZS-A9CF-01	TCGA-ZS-A9CF（2412 Day）
TCGA-ZS-A9CF-02	
……	……

Comment 3. Fig 1 is too ambiguous to explain the design of study. N represents the number of HCC samples in second line box, whereas the number of genes in other boxes. Identify the number of HCC cohort of TCGA and ICGC in each step. Use different abbreviation for the number of genes.

Reply: Thank you very much for your valuable comments. Here, we apologize for not being

able to explain clearly the design of our study. As suggested by the reviewer, we have revised the flow chart to show clearly the analytical process of this study. In the revised flowchart, "N" represents the number of samples from the TCGA, ICGC, and ESTIMATE databases, and the "n" represents the number of genes. (see revised flowchart) Thank you again for your helpful comments.

Changes in the text: see revised flowchart

Comment 4. Did authors identify the FRGs with independent prognostic value in whole TCGA HCC cohort or TCGA HCC cohort with immune score? If the former is right, the third right box (N=24) may be derived from TCGA, not via immune score. There is no clear explanation about that step in this manuscript.

Reply: Thank you very much for your helpful comments. Here, we apologize for not being able to explain clearly this step in this manuscript.

In this study, a total of 365 samples containing both immune score and survival time were used to screen for differentially expressed FRGs and to construct ferroptosis-based prediction model. Based on these 365 samples, we first screened 22 FRGs that differentially expressed between high- and low-immune scores. Then, we conducted univariate Cox regression analysis and confirmed 24 FRGs that were significantly associated with overall survival in HCC patients. There findings were also based on the 365 samples containing both immune score and survival time. As suggested by the reviewer, we have revised the manuscript to clearly explain how to screen out the 24 FRGs with independent prognostic value. (see Page 10, line 214-215) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 10, line 214-215

Comment 5. Include the name of 22 ferroptosis related DEG in result section.

Reply: Thank you very much for your valuable comments. Here, we apologize for not being able to list the name of 22 ferroptosis-related DEGs in "Results" section. As suggested by the reviewer, we have added the name of 22 ferroptosis-related DEGs in "Results" section. (ABCC1, ACSF2, ACSL4, ALOX5, CD44, CRYAB, DPP4, FTH1, G6PD, GCLC, HMGCR, HMOX1, HSBP1, NCOA4, NFS1, PEBP1, PGD, PHKG2, SAT1, SLC1A5, SQLE, and

TP53) (see Page 10, line 212-214) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 10, line 212-214

Comment 6. To enhance the understanding of readers with biological background, author should explain Fig. 2D and 2E in detail. Suggest the meaning about log lambda, coefficient, and partial likelihood ~ and rational interpretation.

Reply: Thank you very much for your valuable comments. Here, we apologize for not being able to explain in detail the meaning of some important parameters in Figure 2D and Figure 2E. As suggested by the reviewer, we have added some sentences to explain what these parameters mean. (see Page 7, line 148-149; see Page 24, line 514-523) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 7, line 148-149; see Page 24, line 514-523

Comment 7. Is there any statistic value for Fig. 3B and 4B? It looks like that the two groups did not show any difference in survival time.

Reply: Thank you very much for your valuable comments. Here, we apologize for the confusion caused by the Figure 3B and Figure 4B.

In this study, we draw Figure 3B and Figure 4B mainly to show the distribution of survival status and survival time of each sample in the training cohort and validation cohort, respectively, and cannot give a clear conclusion on whether there is a statistical difference. In addition, another reason why we draw these two figures is to refer to the analysis ideas of most previous articles that build predictive models based on bioinformatics, that is, they also include such figures. In this study, in order to explore whether there is a survival difference between the high- and low-risk groups, we plotted Figure 3E and Figure 4E to show the results of Kaplan-Meier survival analysis.

Here, we sincerely thank you for constructive comments. We also realize the necessity to validate these results of Kaplan-Meier analysis by larger cohort studies. To verify these findings, we are currently in the process of collecting clinical samples. The lack of a large validation cohort is a flaw in this study. In the revised manuscript, we have added a description of the defect that this study failed to be verified by a larger cohort. (see Page 18,

line 382) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 18, line 382

Comment 8. The title of Fig. 4 would be "~ in ICGC cohort", not "~ in TCGA cohort".

Reply: Thank you very much for your review. Here, we apologize for not being able to find this writing error. As suggested by the reviewer, we have modified this writing error. (see Page 24, line 531) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 24, line 531

Comment 9. Font size in ALL of figures are too small. It is illegible for Fig. 6~9. It is barely hard to recognize whether it is star (significance) of letter (maybe NS?) in Fig. 8.

Reply: Thank you very much for your valuable comments. Here, we apologize for the fact that the fonts in these figures are too small for the content to be read clearly. As suggested by the reviewer, we have re-modified the font size of these figures to ensure that the contents of the figures are clearly displayed. (see revised Figure 6-9) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see revised Figure 6-9

Comment 10. page 17, line 371 spacing: geneand -> gene and

Reply: Thank you very much for your review. Here, we apologize for not being able to find this writing error. As suggested by the reviewer, we have corrected this writing error. (see Page 26, line 569) Thank you again for your time and effort reviewing our manuscript.

Changes in the text: see Page 26, line 569