Peer Review File

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Reviewer A

The paper titled "Copper Metabolism related lncRNAs Predicts Prognosis and Immune Landscape in Liver cancer Patients" is interesting. The prognostic signature consisted of 4 CMRLs proves an outstanding predictive performance and improves the precision immuno-oncology. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the metabolic features, mutation signatures, and immune profile of CMRLs-classified HCC patients? What are the role of CMRLs in therapy guidance? It is recommended to add relevant content.

Reply 1: Thank you for your comment. The metabolic features are primarily reflected in the alteration of copper homeostasis, leading to oxidative stress and cellular toxicity, which are involved in tumorigenesis. We have added new references in the introduction section to explain this further. Regarding the mutation signatures and immune profile, previous studies did not find conclusive evidence. However, our results indicate that in the high-risk groups, TP53 (39%) and CTNNB1 (30%) had higher mutational rates (Figure 10A), while TTN (23%) was higher in the low-risk groups (Figure 10B). Our study suggests that CMRLs may influence the response of HCC patients to immunotherapy by regulating the expression and function of macrophages (Figure 8B). These findings provide robust theoretical evidence filling the gap in this area of research. Previously, there was a lack of research on CMRLs in therapy guidance. Our study confirmed the potential of CMRLs in guiding personalized immunotherapy and selected different drugs for patients in different high-risk groups.

Changes in the text: we added metabolic features of Copper Metabolism related genes, see Page 2, line 82-85.

2) Please puzzle and integrate the figures according to the magazine's requirements.

Reply 2: Thank you for your comment. In the revision of figure, we have already puzzle and integrate the figures according to the magazine's requirements.

3) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply 3: Thank you for your valuable feedback. Despite the known significance of copper in various physiological and pathological processes, there is a notable lack of comprehensive studies investigating the role of CMRLs specifically in the HCC. Existing research primarily focuses on other cancer types or cuproptosis, leaving a substantial gap in our understanding of how CMRLs influences HCC initiation, progression, and metastasis. We added this to the introduction of the revised manuscript.

Changes in the text: we added knowledge gaps and limitations of prior study and the clinical significance of this study, see Page 2 and Page 3, line 87-90 and 94-97.

4) The description of some methods in this study is too simplistic, please describe in detail.

Reply 4: Thank you for your comment. We have described some methods in our study in detail to make it more comprehensive. In the section "2.2 The Differential Expressed Analysis and Construction of the Prediction Signature of CMRLs," we added explanations on eliminating overfitting, conducting multivariate analysis to select genes, and calculating their correlation coefficients. In "2.4 Independent Prognostic Evaluation and Innovative Nomogram Design," we provided additional details on the R language packages used for calculating the C-index. In "2.7 Drug Sensitivity of the Signature," we supplemented information on the drug database. In "2.8 Analysis of Somatic Mutations," we included the methodology for calculating the tumor mutation burden (TMB) score.

Changes in the text: we added detailed method description, see Page 3, line 116-118, see Page4 line 137 and 159, see Page5 line 164-166.

5) What are the roles of CMRLs in predicting immunotherapy response? It is recommended to add relevant contents.

Reply 5: Thank you for your comment. Research on copper metabolism in the context of immunotherapy remains significantly underexplored, with particularly scarce studies focusing on HCC. However, it has been demonstrated that copper metabolism regulates the biological behavior of tumors by maintaining intracellular copper homeostasis. Our study further screened immunotherapy drugs and supplemented the understanding of potential mechanisms related to copper metabolism. We believe that our research provides valuable insights and a robust theoretical basis for future investigations, and paves the way for developing novel therapeutic

strategies that leverage copper metabolism to enhance immunotherapy efficacy in liver cancer patients.

6) The biological characteristics of CMRLs and its research progress in tumors should be added to the discussion.

Reply 6: Thank you for your comment. We have further discussed the biological characteristics of CMRLs and its research progress in tumors

Changes in the text: we added the discussion, see Page9 line 341-353.

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Comprehensive analysis of cuproptosis-related long noncoding RNA immune infiltration and prediction of prognosis in patients with bladder cancer, Front Genet, PMID: 36186475". It is recommended to quote this article.

Reply 7: Thank you for your comment. We have cited the paper titled: Comprehensive analysis of cuproptosis-related long noncoding RNA immune infiltration and prediction of prognosis in patients with bladder cancer in the part of my introduction to make it more comprehensive.

Changes in the text: we added the reference, see Page3 line90.

8) This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.

Reply 8: Thank you for your valuable feedback and recommendations regarding our study. We appreciate your suggestion to incorporate in vivo and in vitro experimental studies to enhance the robustness and applicability of our bioinformatics analysis. Unfortunately, due to current constraints, we are unable to conduct these experiments at this time. However, we fully recognize the importance of such experimental validation and are committed to undertaking these studies as soon as conditions allow. We plan to perform comprehensive in vivo and in vitro experiments in future work to further validate and support our bioinformatics findings. We hope this addresses your concerns, and we appreciate your understanding.

Reviewer B

1. Figures
   - Figures and should be cited **consecutively** in the text and numbered in the order in which they are discussed. Therefore, Figure 7B should be cited before Figure 7C; Figure 7D should be cited before Figure 7E. Please check through and revise.

Reply: Thank you for your comments. We have revised the figure numbers and cited them accordingly. The corresponding changes have been reflected in the text and figure legends.

Changes in the text: Please see Page 7, line284, 286-288.

- There is no Figure 8H in Figure 8. And the citation of Figure 8G is missing. Please check and revise.

tumor immune microenvironment, showing the higher immune levels and immunogenicity of the tumor microenvironment (Figure 8H).¶

Reply: Thank you for your comments. We apologize for mistakenly labeling Figure 8G as Figure 8H. The corresponding error has been corrected in the text.

Changes in the text: Please see Page 8, line311

- **All abbreviations** in figures/tables and legends should be explained. DEGS, LIHC, CMRL in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legends/footnote. E.g., Figure 1. xxx. **Abbreviations: xxx, xxxx**.

Reply: Thank you for your comments. We have been explained all abbreviations in figure and legend.

Changes in the text: Please see document named Figure.

- Figure 1A: Please indicate the meaning of the **green**, **red** and **black dots** in the legends.

Reply: Thank you for your comments. We have been indicated the meaning of the green, red and black dots in the legends.

Changes in the text: Please see document named Figure, Page 1.

- Figure 1E, 6A-6B: Please revise pvalue to **P Value**; Hazard ratio to **Hazard Ratio (95% CI)**.

Reply: Thank you for your comments. We have been revised pvalue to P Value and Hazard ratio to Hazard Ratio (95% CI).

Changes in the text: Please see document named Figure, Page 1 and Page 4.

- Please provide Figure 1F in **higher resolutions** if possible.

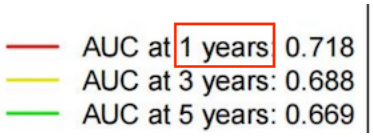
Reply: Thank you for your comments. We have been provided Figure 1F in higher resolutions.

Changes in the text: Please see document named Figure, Page 1.

- Figure 2A-B: Please check if the following word should be **score**. Please also check through your figures to avoid spelling mistake.

Reply: Thank you for your comments. We have been corrected socre to score and checked through our figures to avoid spelling mistake.

- Figure 2G-2I: Please revise 1 years to **1 year**.

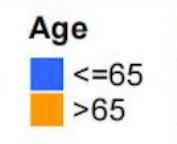


Reply: Thank you for your comments. We have been revised 1 years to 1 year.

- Figure 4A: Please indicate the meaning of * and **.

Reply: Thank you for your comments. We have been indicated the meaning of *, **, and *** in the figure legend of Figure 4.

- Figure 4A-4B, 5A-5B: Please add a unit to the age, and revise <= to ≤.



Reply: Thank you for your comments. We have been added a unit to the age and revised <= to ≤ of Figure 4 and Figure5.

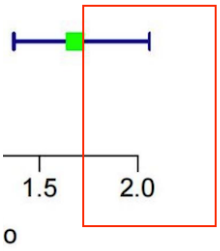
- Figure 4A: Please check if it should be **unknown**.

Reply: Thank you for your comments. We have been corrected unknow to unknown.

- Figure 4B-4E: Please check if % should be added to the y-axis.

Reply: Thank you for your comments. We have been added % to the y-axis.

- Figure 6A, 6B: Please extend the x-axis.

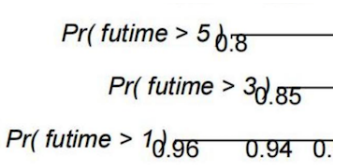


Reply: Thank you for your comments. We have extended the x-axis.

- Figure 6C: Please indicate the meaning of *** in the legends.

Reply: Thank you for your comments. We have indicated the meaning of *** in the legends.

- Figure 6C: Some of the words are overlapped. Please check if it's necessary to revise.

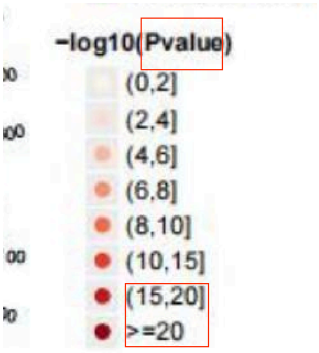


Reply: Thank you for your comments. We have revised the Figure 6C to reduce overlap.

- Figure 6F: Please check if **%** should be removed since the axis range from 0-1.

Reply: Thank you for your comments. We have removed % from the axis.

- Figure 7A-B: Please revise Pvalue to P value. Also, please check if 20 was **repeated** in the following ranges.

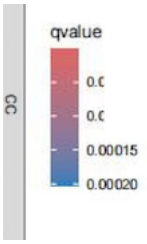


Reply: Thank you for your comments. We have revised Pvalue to P value and corrected the repeated 20.

- Figure 7C-7F: Please revise qvalue to **q value**.

Reply: Thank you for your comments. We have revised qvalue to q value.

- Figure 7E: The following words are **incomplete**. Please check and revise.

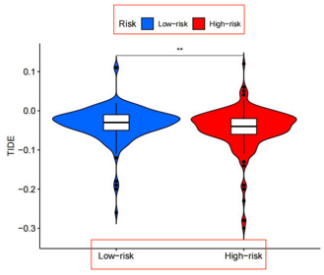


Reply: Thank you for your comments. We have revised the incomplete words.

- Please provide Figure 8A in higher resolutions.

Reply: Thank you for your comments. We have provided Figure 8A in higher resolutions.

- Figure 8F, Figure 9, Figure 10C: The symbols are **repeated**. Please check and revise.

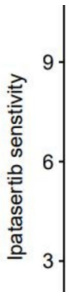


Reply: Thank you for your comments. We have revised the repeated symbols in the Figure 8F, Figure 9, and Figure 10C.

- Figure 9: Please revise all senstivity to **sensitivity**.

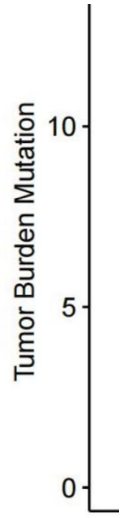
Reply: Thank you for your comments. We have revised the all senstivity to sensitivity in Figure 9.

- Figure 9H: Please check if the description of y-axis should be **Lpatasertib sensitivity**.



Reply: Thank you for your comments. We have revised the lpatasertib sensitivity to Lpatasertib sensitivity in Figure9H.

- Figure 10C-10D: Please check if the description of the y-axis should be **Tumor Mutational Burden**.



(C) TMB between high-risk and low-risk patients. (D

Reply: Thank you for your comments. We have revised the wrong description of the y-axis to be Tumor Mutational Burden in Figure 10C-10D.

2. References 24 and 28, 30 and 32, 8 and 17 are **repeated**. Please check and revise.

- 24. Chang W, Li H, Zhong L, et al. Development of a copper metabolism-related gene signature in lung adenocarcinoma. Front Immunol. 2022;13:1040668. Published 2022 Nov 29. doi:10.3389/fimmu.2022.1040668

  28. Chang W, Li H, Zhong L, et al. Development of a copper metabolism-related gene signature in lung adenocarcinoma. Front Immunol. 2022;13:1040668. Published 2022 Nov 29. doi:10.3389/fimmu.2022.1040668

- 30. Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification. Adv Cancer Res. 2021;149:1-61. doi:10.1016/bs.acr.2020.10.001

  32. Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification. Adv Cancer Res. 2021;149:1-61. doi: 10.1016/bs.acr.2020.10.001. Epub 2020 Nov 28. PMID: 33579421; PMCID: PMC8796122.

- 8. J iang Y, Huo Z, Qi X, Zuo T, Wu Z. Copper-induced tumor cell death mechanisms and antitumor theragnostic applications of copper complexes. Nanomedicine (Lond). 2022;17(5):303-324. doi:10.2217/nnm-2021-0374

  17. Jiang Y, Huo Z, Qi X, Zuo T, Wu Z. Copper-induced tumor cell death mechanisms and antitumor theragnostic applications of copper complexes. Nanomedicine (Lond). 2022;17(5):303-324. doi:10.2217/nnm-2021-0374

Reply: Thank you for your comments. We have revised the repeated references.

3. Please check if any reference should be added since you mentioned the author's name.

expression differences in high and low risk groups. List of genes positively correlated with anti PD-L1 drug response obtained from Xu et al's cancer and immunity website (http://biocc.hrbmu.edu.cn/TIP/) and Mariathasan's research features were enriched with biological signal features favorable for cancer immunotherapy in low-risk and high-risk groups using the

Reply: Thank you for your comments. We have added related references which mentioned the author's name in our research.

Changes in the text: Please see Page 5, line198.