Point by point response to the Reviewers and Editors

We appreciate the insightful comments made by all the reviewers and editors. Below are the point-by-point responses to each specific comment. The **BLACK** fonts are from reviewers' and editors' comments, and the **GREEN** fonts are our point-by-point responses.

Reviewer #1:

The article looks interesting, but I have a few concerns

We thank the reviewer for the summary of this study.

1. The rationale for choosing the topics should reflect in the introduction part

The reviewer's suggestions are gratefully accepted. We added the following sentences to highlight the rationale for the selection of the topic in the second paragraph of the Introduction section when introducing copper metabolism and cuproptosis: "Considering the double-edged function of copper, which is an essential enzyme cofactor but also produces toxicity that causes cell death, copper is expected to be a new therapeutic target used to specifically kill cancer cells by increasing intracellular copper accumulation.... These all indicate the great potential of copper in antitumor therapy for cancers that are naturally resistant to apoptosis. Given that lipid acylation, a major target of cuproptosis for cytotoxicity, is widespread and conserved in nature, the use of this metabolic profile for copper ion-targeted therapy of tumors is promising and there is an urgent need for reliable and accurate detection of biomarkers of cuproptosis in human tumor tissues." (See INTRODUCTION Section, Paragraph 2).

2. How do you construct the co-expression network of cuproptosis-related genes and lncRNAs ? need more clarity for general readers.

We thank the reviewers for their comments. To provide more clarity to the general readers, we have added the following sentence to the MATERIALS AND METHODS section: "Based on gene names, transcriptional expression profile data were classified as lncRNA or mRNA, and lncRNAs associated with 19 cuproptosis-related genes (mRNAs) were identified using the "limma" package and the Pearson correlation test." We believe that this plus subsequent

sentences can provide enough information to the general reader to illustrate how we constructed the mRNA-lncRNA co-expression network (See MATERIALS AND METHODS Section, Paragraph 2).

3. How do you plan for cuproptosis-related lncRNA signature analysis, taken help from Bio-statistician or any software statistical analysis?

We sincerely thank the reviewers for their excellent comments. All the analysis in this manuscript were done using various packages in the R software. The statistical applications built into these packages have been validated with a large amount of data, and their integrated application in bioinformatics analysis has been reported in a large body of literature. Therefore, the statistical methods used in the present study are mature and robust, which can well support our conclusions. The statistical methods in this study, the R packages, the figures they produced, and the literatures that used them are partly listed in the table below. Finally, the statistical methods used in this study were reviewed by Ganfeng Luo, PhD (ORCID: 0000-0003-2043-4554), a graduate in epidemiology and health statistics from the School of Public Health, Sun Yat-sen University, Shenzhen, China.

Statistical methods	**R packages**	**Figures generated by the specific package**	**Literature that uses the package**
univariate Cox regression	survival	Figure 2B	(1)
Lasso regression	glmnet	Figure 2C, 2D	(2)
Pearson's correlation analysis	limma	Figure 2E	(3)
log-rank test	survival	Figure 3A-C	(4)
c-index	rms, pec	Figure 4B	(5)
T test	maftools, TIDE	Figure 9C, Figure 11B	(6,7)
one-way ANOVA	survminer,	Figure 9D, 9E	(6)

test	survival		
Wilcoxon rank-sum test	CIBERSORT, GSVA, limma, reshape2, pRRophetic	Figure 10A, Figure 10B, Figure 11A, Figure 11C-J	(1,8-10)

4. The diagrams need clarity, 300dpi resolution is preferred

Many thanks to the reviewers for their comments. We replaced all the images to 300 dpi format and attached them to the .pptx file uploaded.

Reviewer #2:

The researchers believe that the lncRNA signature, CupRLSig, is valuable in prognostic estimation in the setting of HCC. Importantly, CupRLSig likely also predicts the level of immune infiltration and potential efficacy of tumor immunotherapy, chemotherapy, and targeted therapy. The study does have some value as stated by the authors, and the figures and tables in the manuscript are of good quality. But I think the following questions still need to be addressed to highlight the clinical value of this study.

We thank the reviewer for the in-depth summary of this study.

1. The Results section in the manuscript lacks an introduction to the basic characteristics of patients

The reviewer's suggestions are gratefully accepted. We added some sentences to describe the basic characteristics of patients in the Result Section as follows: “We collected 343 samples with complete clinicopathological data from the TCGA-LIHC database. The characteristics and materials were from 233 males and 110 females, 224 of whom are still alive. The age of HCC patients ranged from 16 to 90 years, and their survival after diagnosis ranged from 30 to 3,675 days. More specific characteristics are shown in Table 1.” (See RESULTS Section, Paragraph 1).

2. Is the implementation of cuproptosis-related lncRNA signature feasible in clinical practice?

We thank the reviewers for their comments. Despite the need for additional external validation set verification, our current findings indicate that the cuproptosis-derived lncRNA signature has a high potential for use as a biomarker in clinical practice. First, it is generally acknowledged that including multiple biomarkers in a single model rather than just one biomarker can increase the prognostic prediction's accuracy (8). Additionally, because the technology is more readily available and is becoming less expensive, bulk-sequencing is a technique that may more easily be introduced into the clinic as a standard management method. We therefore believe that we can expand the bulk-sequencing-generated lncRNA model to the standard care of HCC patients if sufficient external data is available, which can validate the predictive efficacy of cuproptosis-related lncRNAs.

3. It is mentioned in the text that the low-risk group had more activated natural killer cells (NK cells, $p = 0.032$ by Wilcoxon rank sum test) and fewer regulatory T cells (Tregs, $p = 0.021$) infiltration than the high-risk group. Then can the use of immune cells also predict patient prognosis? The use of monitoring immune cells to reflect the patient's condition and prognosis seems more in line with clinical applications.

We totally concur with the reviewers and value their thoughtful analysis of how immune cells are utilized to determine patient prognosis. Based on the fact that immune cells are indeed known to be a prognostic guiding biomarker (11), we used the different distribution of immune cells to explain the potential mechanisms of prognostic differences between the high- and low-risk groups. However, accurate immune cell quantification in tumor tissues is in fact not feasible in clinical practice at this moment due to the limitations of accessibility and high cost of flow cytometry, multiplex immunohistochemistry, or single cell sequencing. In contrast, bulk-sequencing combined with a deconvolution algorithm to indirectly quantify the immune content in tumor tissues is easier to implement in clinical practice after more external samples have been tested and replicated for our lncRNA model. The design of this study is based on this theory, using data from bulk-sequencing to infer the level of immune cells, which in turn reflects the patient's condition and prognosis.

4. The article discusses the immunotherapy of patients with different risk levels, so is it applicable to the early and late stages of patients with different risk levels?

We sincerely thank the reviewers for their excellent comments and share their concern about the different risk levels and sensitivities of early- and late-stage patients to immunotherapy. This may be due to the fact that a large proportion of currently approved immunotherapeutics are used for the back-line treatment of metastatic disease. In contrast, first-line systemic therapy for early- and mid-stage tumors remains dominated by chemotherapy and targeted therapy, except for patients with specific positive molecular markers, such as microsatellite instability-high.

In terms of the study itself, because TCGA-LIHC includes both early (I-II) and late (III-IV) stage patients, our model was generated based on these data and therefore naturally predicts the outcome of immunotherapy in HCC patients, both early- and late-stage. Of course, we are very aware that the model needs to undergo some external validation. As far as we know, except for TCGA, HCC has few other public datasets with such a large sample size and complete prognostic data available, as opposed to breast cancer (METABRIC and ICGC datasets) and glioma (CGGA datasets). Even though a relatively large HCC sample has prognostic data available in the GEO database, they often used the coding gene array platform rather than the next generation sequence (NGS) or lncRNA array platforms. As a result, we were unable to validate our TCGA-generated lncRNA model on even one external HCC dataset at present. However, when additional external data is available, it would be considered to determine whether the HCCSenLncSig model adequately fits the specific dataset.

5. The ethics number seems to be missing from the text.

Many thanks to the reviewers for their comments. Since we are not using any data from animal research or our own clinical samples in this bioinformatics work, there is no requirement for ethical permission from our institution or an ethics number. However, thanks to the kind reminder of the reviewers, we have added a statement in the MATERIALS AND METHODS Section and the Footnotes Section: The study was conducted in accordance with

the Declaration of Helsinki (as revised in 2013) (See MATERIALS AND METHODS Section, Paragraph 1 and Footnotes Setiocn, Paragraph 1.)

Science editor:

According to both reviewers, this manuscript needs polishing in English. Besides, the design of this study, the rationale to select the candidate molecular signature, the background of the study needs to be improved. The quality of figures should fulfill the requirements of this journal.

Many thanks to the science editor for their comments. The language in this article has been edited by Charlesworth Author Service once more, and some changes have been made to the study design, context, and topic selection. Please refer to the above point-by-point response to the reviewers for changes. The quality of the figures was improved, specifically by integrating the 300 dpi resolution figures into a single .pptx file and uploading it again to the submission system.

Company editor-in-chief:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastrointestinal Oncology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the

keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/. Uniform presentation should be used for figures showing the same or similar contents; for example, “Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...”. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file.

Many thanks to the editor-in-chief for his/her comments. We provide decomposable figures with 300 dpi resolution that are integrated into a single .pptx file. Additionally, we added a citation (Reference (4) by Zhang et al.) based on the RCA search results （In the manuscript is the **Reference (11)** ）.

Reference

1. Chen M, Nie Z, Li Y, et al. A New Ferroptosis-Related lncRNA Signature Predicts the Prognosis of Bladder Cancer Patients. Front Cell Dev Biol 2021;9:699804.
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4. Zhang G, Sun J, Zhang X. A novel Cuproptosis-related LncRNA signature to predict prognosis in hepatocellular carcinoma. Scientific Reports 2022;12:11325.
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6. Xu M, Lu J-H, Zhong Y-Z, et al. Immunogenic Cell Death-Relevant Damage-Associated Molecular Patterns and Sensing Receptors in Triple-Negative Breast Cancer Molecular Subtypes and Implications for Immunotherapy. Frontiers in oncology 2022;12:870914-.
7. Jiang P, Gu S, Pan D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nature medicine 2018;24:1550-8.
8. Guo Y, Qu Z, Li D, et al. Identification of a prognostic ferroptosis-related lncRNA signature in the tumor microenvironment of lung adenocarcinoma. Cell Death Discovery 2021;7:190.
9. Liang L, Yu J, Li J, et al. Integration of scRNA-Seq and Bulk RNA-Seq to Analyse the Heterogeneity of Ovarian Cancer Immune Cells and Establish a Molecular Risk Model. Frontiers in Oncology 2021;11.
10. Wang Z, Wang Y, Yang T, et al. Machine learning revealed stemness features and a novel

stemness-based classification with appealing implications in discriminating the prognosis, immunotherapy and temozolomide responses of 906 glioblastoma patients. Briefings in Bioinformatics 2021;22.
11. Mlecnik B, Bindea G, Angell HK, et al. Integrative Analyses of Colorectal Cancer Show Immunoscore Is a Stronger Predictor of Patient Survival Than Microsatellite Instability. Immunity 2016;44:698-711.