Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-23-412

Reviewer A

Colorectal cancer (CRC) remains the most common gastrointestinal malignancy. Despite multimodal therapy, its mortality is high due to recurrence and metastasis. In the manuscript "A risk model constructed using 14 N6-methyladenosine-related lncRNAs as a new prognostic marker that correlates with the immunomodulatory effect and drug sensitivity in colorectal cancer", authors developed and verified a risk model consisting of 14 N6-methyladenosine (m6A) long noncoding RNAs (lncRNAs) to assess the prognosis of patients with CRC and investigated its relevance to immune regulation and drug sensitivity.

Couple questions are required to be answered before it will be accepted.

Comment 1: The m6A and lncRNA was the crucial topic in the study. What were the correlations between m6A and lncRNAs? Please state in the introduction.
Reply 1: Please accept our thanks for your work. For the content of this section, actually, we made some statements in the introduction. The significance of m6A in lncRNA was described on pages 5, lines 107-109 of our manuscript. Furthermore, based on your suggestion, we have added some statements in the introduction.
Changes in the text: We have added some statements on page 5, lines 109-115.

Comment 2: In the introduction, it was better to add related reference (DOI: 10.21037/jtd-22-1185) about the prognostic model with m6A lncRNA.
Reply 2: We have added it in the introduction.
Changes in the text: We have added it on page 5, lines 113-115.

Comment 3: What were the roles of m6A in the immunoregulation and drug sensitivity? Please state in the introduction.
Reply 3: Thank you very much for your comments. Based on your suggestion, we have added some statements in the introduction.
Changes in the text: We have added it on page 4, lines 91-94.

Comment 4: Missing experimental data was the biggest short board. It was better to validate the crucial lncRNA and drug sensitivity by experiments.
Reply 4: In this study, our aim is to identify m6A related lncRNAs that may play an important role in colorectal cancer, which is a preliminary exploration. We will analyze the sensitivity of key molecules and drugs in future research progress, which is also our future outlook. (See page 17, lines 534-536).
Changes in the text: Not Applicable.

Comment 5: In the figure 3, please mark the A1-A4.
Reply 5: We have added the corresponding markers in the Figure 3. (See Figure 3-revised).

Changes in the text: (See Figure 3-revised).

Comment 6: The immuno-reactivity was analyzed in the study. It was better to analyze the effects on infiltrated immune cells.

Reply 6: Thank you very much for your kind work. In this study, we focused more on the correlation with clinical immunotherapy applications. Our results suggest that the constructed model may be related to immune function, and further analyzed its TIDE score and added an assessment of the TMB (which has been considered a marker of immunotherapy in recent years) and found that it can be used as a helpful prognostic predictor in conjunction with our model. For the impact of infiltrating immune cells, we will conduct further research on a specific key molecule in future mechanisms.

Changes in the text: Not Applicable.

Comment 7: Compared to other constructed prognostic model, what were the advantages of constructed model with m6A lncRNAs for CRC? Please state in the discussion.

Reply 7: Please accept our sincere thanks for the comments provided by you. Based on your suggestion, we have added some statements in the discussion.

Changes in the text: We have added it on page 14, lines 441-444, and page 16, lines 509-525.

Reviewer B

Comment 1: First of all, my major concern is the risk score based on 14 N6-methyladenosine-related lncRNAs is only one of the predictors in the nomogram to predict the prognosis, but the title did not clearly indicate this. My second major concern is the unsatisfactory predictive accuracy of the nomogram proposed with AUC values between 0.715-0.763, which did not support "could accurately predict the prognosis of patients with CRC". The third concern is the unclear descriptions of the generation of training and validation samples and how the predictive external validity was verified. The authors also did not describe the research design in the title such as a bioinformatics analysis.

Reply 1: First, this study excavated the 14 prognostic m^6A-related lncRNAs to construct a risk model. Our subsequent research work revolved around this risk model rather than the nomogram. The nomogram is a tool for applying the constructed risk model to clinical practice and is not the subject of this study.

For your second concern, according to the judgment criteria, when the AUC value is greater than 0.7, there is a certain degree of accuracy. Therefore, the results of this study are valid, but they did not reach the level of accuracy. So, we greatly appreciate your rigor and have made changes to the manuscript regarding this aspect, removing the expression of "accurately".

For your third concern, in our study, we randomly assigned patients to the training and test groups at a 1:1 ratio, and the attached trilinear table compares the information on patients included in both groups, which is sufficient to demonstrate comparability. We have added these descriptions to page 6, lines 154 - 156. We appreciate your assistance in making this correction. In regard to external validation, we were actually more eager to have external data available to validate the model developed in this study. Despite our efforts to search databases

such as GEO (GSE37892, GSE39582, GSE17538, GSE110223, GSE17536, GSE110224, GSE153127, GSE206613, GSE1626667, GSE106584, GSE156720, GSE175433), ICGC, etc., we have not been able to locate any suitable data for this study due to inconsistencies in public databases and the absence of lncRNA data. Based on this consideration, we randomly assigned patients to the training and test groups at a 1:1 ratio, and the attached trilinear table (Table 1) compares the information on patients included in both groups, which is sufficient to demonstrate comparability. Furthermore, we extended it by including a whole cohort of patients for double validation. This practice is common in published studies without an external validation set, and their results are well supported [1-3].

[1] Jiang D, et al. Development of genomic instability-associated long non-coding RNA signature: A prognostic risk model of clear cell renal cell carcinoma. Front Oncol. 2022 Oct 19; 12:1019011.

[2] Jiang P, et al. A new acidic microenvironment related lncRNA signature predicts the prognosis of liver cancer patients. Front Oncol. 2022 Oct 31; 12:1016721.

[3] Guo S, et al. Identification and validation of ferroptosis-related lncRNA signature as a prognostic model for skin cutaneous melanoma. Front Immunol. 2022 Sep 29; 13:985051.

Regarding your opinion to add a statement of bioinformatics in the title, as our research focus is on identifying key prognostic m^6A-related lncRNAs, rather than some innovation in bioinformatics technology. Therefore, I believe this may not be necessary.

Changes in the text: We have modified our text as advised. (See page 2, lines 54) and added some descriptions to page 6, lines 154 - 156.

Comment 2: Second, the abstract needs further revisions. The background did not explain why 14 m6A-related lncRNAs are associated with prognosis in CRC, why it can accurately predict the prognosis, and what the potential clinical significance of this research focus is. The methods need to describe the generation of training and validation samples, potential predictors, and prognosis outcomes to be predicted. The results need to describe the predictors in the nomogram and AUC values for its predictive accuracy. Please also quantify the findings by using statistics such as survival rates and accurate P values. The conclusion needs detailed comments for the clinical implications of the findings.

Reply 2: As shown in the title of this article, the research subject of this article is the risk model constructed using 14 m^6A-related lncRNAs. Our subsequent research work revolved around this risk model rather than the nomogram. The nomogram is a tool that combines independent prognostic factors including the risk model to predict patient survival outcomes in clinical practice. And its reliability had been verified through our calibration curves and examples. We constructed a risk model based on 14 m6A-related lncRNAs that could predict the prognosis of patients with CRC and provided additional therapeutic ideas for their treatment. These findings may additionally serve as a foundation for further studies on regulating CRC via m6A-related lncRNAs.

Changes in the text: Not Applicable.

Comment 3: Third, in the introduction of the main text, the authors did not review known prognostic biomarkers in CRC, did not analyze their limitations, and did not explain the

potential strengths of 14 m6A-related lncRNAs as accurate prognostic predictors in CRC. Please clearly describe the clinical significance of this research focus.

Reply 3: In the third and fourth paragraphs of the introduction, we reviewed the important significance of m6A and lncRNA in colorectal cancer, respectively. Based on your suggestion, we have added some statements of m^6A-related lncRNAs in cancer progression in the fifth paragraph.In our study, the risk model we have constructed is more comprehensive than other models and has proven to be a superior predictor of prognosis for colorectal cancer patients compared to other factors.

Changes in the text: We have added some statements on page 5, lines 107-116.

Comment 4: Fourth, in the methodology of the main text, please describe the research design, have an overview of the research procedures and the questions to be answered by them, the clinical sample and data in the dataset including the prognosis outcomes, and how the training and validation samples were generated. In statistics, please describe the threshold values of AUCs for a nomogram with good predictive accuracy.

Reply 4: The flowchart in Figure 1A displayed the construction and verification process of the risk model based on the m6A-related lncRNAs and subsequent analyses. (See page 9, lines 251-252). In the Data acquisition and preprocessing section of the methods, we have explained the source of the data (See page 5, lines 130-137). We corrected the relevant statements and explained the generation of the training and validation samples (See page 6, lines 154-156). In this study, our research focus is on the constructed risk model. The nomogram is a tool that combines independent prognostic factors including the risk model to predict patient survival outcomes in clinical practice. And its reliability had been verified through our calibration curves and examples.

Changes in the text: We have modified our text as advised. (See page 6, lines 154-156).

Reviewer C

1. Figure 1

Please explain TCGA, OS, PCA, and LASSO in the legend.

Reply: We have added it on page 22, lines 702-703.

2. Figure 3

In the figure 3, there are not A1, A2..A4, please check.

292 significantly worse prognosis than did those in the low-risk group (Figure 3A1). Risk

295 with the increase in patient' risk scores (Figure 3A2,3A3). Moreover, in the heat maps,

301 a beneficial effect (Figure 3A4). We performed the same analyses as in the training

Reply: We have added the corresponding markers in the Figure 3.

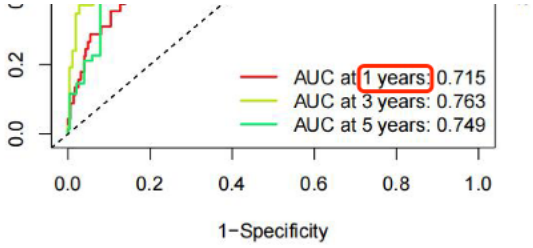
3. Figure 4

a) Gender was missing in the legend, please revise.

778 **Figure 4** Independent prognostic and precision verification analysis of the risk model.
779 (A,B) Univariate and multivariate Cox regression analyses were used to identify age,
780 stage, and risk score as independent factors in the prognosis of patients with CRC. (C)

Reply: Our results indicated that gender was not an independent prognostic factor for colorectal cancer ($p > 0.05$), so there was no need to add a description of gender here.

b) Please revise "1 years" to "1 year" in 4C.

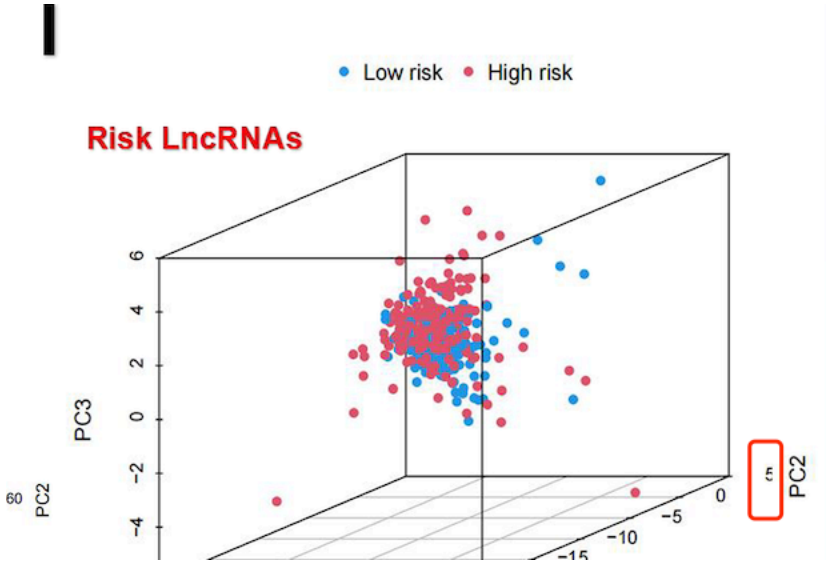


Reply: We have made modifications to it.

c) Please explain AUC in the legend.
Reply: We have added it on page 23, line 734.

4. Figure 5
Please explain the meaning of *** in the legend.
Reply: We have added it on page 23, line 739.

5. Figure 6
The data was covered, please revise.



Reply: We have modified it as advised.

6. Figure 7

a) Please provide a clearer version of 7A.

Reply: We have modified it as advised.

b) As there are no symbols "***" in the figure, please delete the explanations in the legend.

Reply: We have removed the explanation of "***". (See page 24, line 753).