Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-2243

Reviewer A

This article identifies a lncRNA signature that allows to predict endometrial cancer prognosis using a LASSO model.
Reply:
We thank the reviewers for their encouragement and interest in our work. The comments were thoughtful and insightful, and they contributed greatly to improving the quality of our manuscript. In response, we have revised the manuscript. Our revision can be summarized in the following areas:
1. We supplemented the background of lncRNAs related to endometrial cancer and algorithmic prediction of disease models
2. We have added the tumor growth curve and the results of the murine tumor-bearing experiment without adding DOX.
3. In the discussion section, we have added a description of the competitiveness of this model compared to other models and have added the limitations of this manuscript on immune cell testing, and have planned for subsequent experiments.
4. Modification of figure titles, figure legends, and formats.
We sincerely appreciate all the comments brought up by the reviewers. Detailed responses for each comment are below.

Background:
Comment 1: Do the authors know if other lncRNA were linked to endometrial cancer progression in addition to PCAT1?
Reply 1: We thank the reviewer for this comment and have added other lncRNA linked to endometrial cancer progression in the manuscript.
Changes in the text: we have modified our text as advised (see Page 6, line 89-94)

Comment 2: It would be good to provide more background on algorithm prediction for patients with cancer.
Reply 2: We thank the reviewer for this comment and have added background on algorithm prediction.
Changes in the text: we have modified our manuscript as advised (see Page 7, line 102-110)

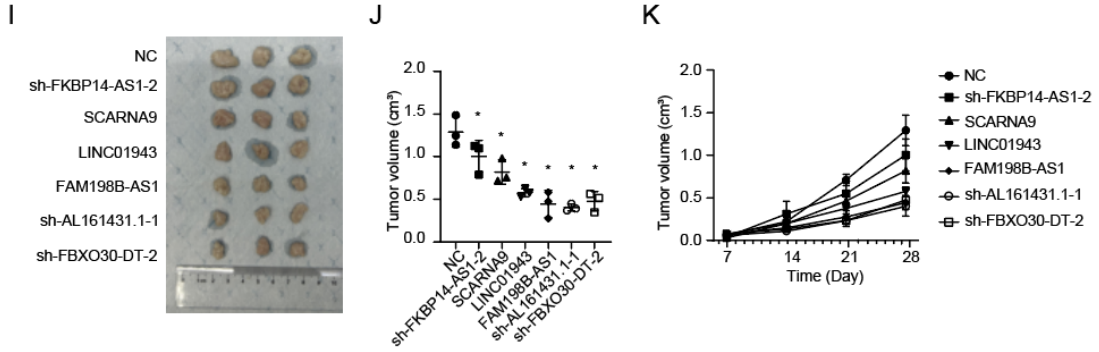
Methods:
Comment 3: It would be interesting to test other predictive models and not only the LASSO one to see if it improves the prediction with this signature.
Reply 3: We thank the reviewer for the insightful comments. LASSO (Least Absolute Shrinkage and Selection Operator) regression is a statistical method used in regression analysis to obtain a model with strong interpretability and good predictability. The use of LASSO regression to screen prognostic biomarkers and construct models for predicting clinical

prognosis outcomes is widely used and accepted in current research. After obtaining the predictive model using LASSO regression, we also used univariate and multivariate Cox regression methods to test the risk ratio of the risk score and validated the reliability using ROC curves (Page 20, line 379-382; Page 21, line 393-394). In vivo and in vitro experiments, we also validated the accuracy of the biomarkers in the model (section 3.9). Your constructive proposal to use multiple prediction models has great inspiration for us, and we will explore it in future research to obtain stronger validation.

Results:

Comment 4: Do the authors know if targeting lncRNA during murine tumor growth without DOX also affects tumor growth?

Reply 4: Thank you for pointing this out. In the preliminary experiment, we targeted lncRNAs without DOX, and the results showed that intervention of the six lncRNAs could inhibit tumor growth to varying degrees (see the attached picture below). After the synergistic application of DOX, we can more intuitively observe the role of these six lncRNAs in the process of immunogenic cell death.



Changes in the text: we have modified our supplementary materials as advised (see Supplementary Figure 7H-7K)

Comment 5: It may also be interesting to investigate the differences of immune infiltration in tumors by flow cytometry.

Reply 5: We thank the reviewer for the insightful comments. Flow cytometry is widely used to test the classification and proportion of immune cells. Our experiment preliminarily analyzed the immune environment through bioinformatics methods and validated immunogenic death using three testing standards. Unfortunately, we have not yet used flow cytometry to further confirm the activation of immune cells. We greatly appreciate your suggestion and have discussed it in the outlook section of the manuscript. In the next step of our project, we will use flow cytometry for in-depth analysis and discussion.

Changes in the text: we illustrated this limitation in the discussion section (see Page 29-30, line 569-575)

Comment 6: Figure 5 and 8 titles are missing.

Reply 6: We are sorry for the loss of the titles. We have added it in the manuscript.

Changes in the text: we added the titles of Figure 5 and 8 (see Page 51, line 959; Page 54, line 991)

Comment 7: On Figure 5 F and 6 C most of the molecules are not immune checkpoints but costimulatory molecules, immunosuppressive molecules and others with different functions. It would be better to group them by their global functions.
Reply 7: Thank you for pointing this out. We have modified Figure 5F and 6C to classify molecules according to their global functions
Changes in the text: we have modified our figures as advised (see Figure 5F and 6C)

Comment 8: It would be better to also indicate the statistical test used in each figure legend and if statistical correction for multiple tests was performed.
Reply 8: Thank you for this suggestion. We have added the indications of the statistical test used in the figure legend.
Changes in the text: we have modified our manuscript as advised (see Page 48, line934-937; Page 49, line 945-947; Page 50, line 955-956; Page 51, line 964-965; Page 52, line 971-973; Page 54, line 987-989; Page 55, line 996-997)

Comment 9: For tumor growth experiments in mice in Figure 8 C/D, it would be better to show growth curves instead of only the end point.
Reply 9: Following the reviewer's comment, we added growth curves.
Changes in the text: we have modified our supplementary files as advised (see Supplementary Figure 7H)

Discussion:
Comment 10: It would be interesting to discuss why this lncRNA signature may be better or complementary with other signatures.
Reply 10: Thank you for pointing this out. We made some comparations with other signatures.
Changes in the text: we have modified our text as advised (see Page 28, line 534-537)

Reviewer B

1. Please type the equation in **MathType** (an equation writer) and copy it into the main body of the text.

   The risk model was as follows:

   $$Risk\ Score = \sum_{i=1}^{n} explncRNA_i \times coeflncRNA_i$$

   Reply: Thank you for your suggestion. We have typed the equation in MathType.
   Changes in the text: we have modified our text as advised (see Page 11, Line 177)

2. Tables and Figures
   1) Please add a unit to "Age" in Table 3.

Reply: Thank you for your suggestion. We have added a unit to “Age”.

Changes in the text: we have modified our text as advised (see Page 49, line 947)

Figure 2

2) Please add a label to indicate the meaning of different color dot in Figure 2B.

Reply: Thank you for your suggestion. We have added labels for different color dot in Figure 2B.

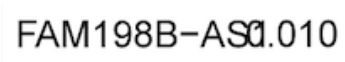
Changes in the text: we have modified our figure as advised (see Figure 2B)

3) Please revise “pvalue” to “P value”, and “Hazard ratio” to “Hazard ratio (95% CI)” In Figure 2C.

Reply: Sorry for the non-standard expression here. We have modified our figure as advised.

Changes in the text: we have modified our figure as advised (see Figure 2C)

4) There has overlapping in Figure 2C, please check and revise.



Reply: Sorry for the overlapping here. We have modified our figure as advised.

Changes in the text: we have modified our figure as advised (see Figure 2C)

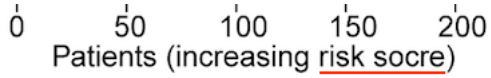
5) Figure 2E has a typo: Positive.

Reply: Sorry for the typo. We have made corrections.

Changes in the text: we have modified our figure as advised (see Figure 2E)

Figure 3

6) Figures 3C-3H: risk socre should be “risk score”.



Reply: Sorry for the typo. We have made corrections.

Changes in the text: we have modified our figure as advised (see Figure 3C-3H)

7) Figure 3N: data are too close to tell, please check and revise.

Reply: Thank you for your suggestion. We have modified our figure as advised.

Changes in the text: we have modified our figure as advised (see Figure 3N)

Figure 4

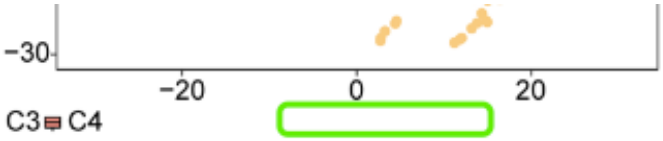
8) Figure 4H: Please define “**” “***”.

Reply: Thank you for your suggestion. We have added definitions of “**” “***” in the figure legend.

Changes in the text: we have modified our text as advised (see Page 54, line 979)

Figure 6

9) Figure 6B: please add the description in the X-axis.



Reply: Sorry for missing the horizontal axis description here We have added the description in the X-axis.

Changes in the text: we have modified our figures as advised (see Figure 6)

Figure 8

10) Please indicate the staining methods in Figure 8A legend and indicate staining methods and magnification for Figure 8E.

Reply: Thank you for your suggestion. We have indicated the staining methods in Figure 8A and 8E legend. We have added magnification for cell maps in Figure 8E legend.

Changes in the text: we have modified our figure legend as advised (see page 59, line 1022, line 1025, line 1026)