Peer Review File

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Reviewer A

Comment 1:

General Comments

The authors' proposal is clear and supports the generation of a new classification system based on genes related to programmed cell death (PCD) in patients with ovarian cancer undergoing surgery. Notably, the authors specifically target treated patients, as the subgroup that does not respond to conventional treatments has a worse prognosis. However, this topic has been addressed by various studies, such as Cai et al. (10.1186/s12885-024-12245-2) and, from another perspective, by Ma et al. (10.1186/s13048-023-01213-2), which unfortunately detracts from the novelty of the proposal. Additionally, the title mentions a multi-omics analytical approach, but the analysis focused only on transcriptomic data associated with clinical metadata.

Reply 1: Thank you for your advices. We have compared the difference and advance between our research and previous articles. In addition, in this research, we applied transcriptomic data and single cell RNA sequence. Previous studies have concentrated on the relationship between cell death and prognosis; however, they have not addressed the heterogeneity of the immune microenvironment in patients with varying prognoses, thereby overlooking the connection between the immune microenvironment and patient outcomes using single cell RNA sequence [38654239；38654239].

Changes in the text: we have modified our text as advised (see Page 20, line 518-525). We also referenced these two articles in our revised manuscript. (see reference 34 and 35)

Comment 2:

Major Comments

The authors must specify what type of transcriptomic data was used in the analyses (TPM, FPKM, FPKM-UQ, etc.) and the kind of normalization used in the microarray validation databases (e.g., z-score normalization, minimums, and maximums, etc.).

Reply 2: Thank you for your advice. We have added this description in methods. To be spetific: The TCGA data were normalized to Transcripts Per Million (TPM), while the microarray datasets were normalized using z-scores. Additionally, all datasets were analyzed using R (version 4.1.1) along with R Bioconductor packages.

Changes in the text: we added some text as advised (see Page 5, line 139-147).

Comment 3:

The authors do not specify the histological type of ovarian cancer evaluated. High-grade serous histological types present a different genomic pattern than mucinous, clear cell, etc., so the results may vary considering the various types of ovarian cancer.

Reply 3: Thank you for your advice. The histological type of ovarian cancer we used in this research was Uterine Corpus Endometrial Carcinoma.

Changes in the text: we added some text as advised (see Page 5, line 131).

Comment 4:

The authors do not specify whether the quality of treatment was considered, that is, whether there was optimal or suboptimal debulking of the patients. These conditions result in completely different prognoses in patients with ovarian cancer.

Reply 4: Thank you for advice very much! We considered this at the beginning of the study, but since we were using public data, not clinically collected cohorts, many cohorts were missing this information. If the cohort is collected clinically, it is likely that such a large number of samples could not be reached, so this is also the defect of our study.

Changes in the text: we added some text as advised (see Page 22, line 553-554).

Comment 5:

The authors analyze genes associated with the formation of tertiary lymphoid structures (TLS) using bulk RNA-seq, which is erroneous. Although common genes can be considered between both areas, the tumor-infiltrating events and cytokine profile are completely different for the tumor microenvironment (TME), so making a direct association without at least single-cell data is impossible. The rest is an overrepresentation of the results.

Reply 5: Thank you for your advice. We feel sorry for the inconvenience brought to the reviewer. In fact, we included single cell RNA dataset to explore the relationship between immune microenvironment and PCD groups in our results: titled “Dissection of tumor microenvironment based on single cell transcriptome”. (see Figure 6 and Figure S3, also see Page 16 line 400-438)

Comment 6:

Figure 2G presents a different coloration, potentially leading to erroneous conclusions.

Reply 6: Thank you for your suggestion and kind reminder. We totally understand the reviewer's concern. Figure 2G shows two different colors, green and purple. Green represents subtype C1 and purple represents subtype C2. We have repeatedly verified and calculated the data represented by different colors, and the results obtained are the same as those in Figure 2G, so we did not make any modifications to this part.

Comment 7:

Many survival analyses are based on generating two groups with significantly different sizes. Therefore, the cutoff is recommended to correspond to the median expression of the gene or score. Please modify this and verify that the findings remain consistent.

Reply 7: Thank you for your advice. We further performed a survival analysis using the median of PCDI and found consistent results (see Figure S3). This proves that our results are credible.

Changes in the text: we added some text as advised (see Page 14, line 366-369).

Comment 8:

Additionally, avoid analyzing survival curves beyond ten years, as this could generate statistical differences that are not comparable to the clinical reality of the patients.

Reply 8: Thank you so much for your careful check. We have deleted patients beyond ten years in survival curves. (see new Figure 4)

Comment 9: Minor Comments

The text mentions the relationship of the PCD cluster with hypoxia, but this result is not observed in Figure 1E.

Reply 9: Thank you for your advice. Hypoxia signaling pathway is significant (NES: 2.04, AdjP < 0.001), but the ranking according to adjP is not in the top ten, so we attach the result of Hallmark channel enrichment (see line 253, Table S2).

Changes in the table: we attach the result of Hallmark channel enrichment in Table S2.

Comment 10:

On line 188, it is stated, “while stage I/II predominated in patients in clusters 1.” However, this is not observed in Figure 1C. Perhaps the authors should change it to Cluster 2.

Reply 10: Thank you for your advice. We are very sorry for our negligence of this point.

Changes in the text: We have changed it to Cluster 2 (see Page 9, line 236)

Comment 11:

The authors could incorporate immune cell estimation using cell deconvolution algorithms, such as CIBERSORT, Ecotyper, xCell, etc. This would enrich the proposal and enable evaluating cellular states associated with survival.

Reply 11： Thank you for your advice. We feel sorry for the inconvenience brought to the reviewer. In this research, we first used single-cell transcriptome data to explore the heterogeneity of the immune microenvironment in the high and low PCDI groups and found that cycling cells had a higher proportion in the PCDI group. We then use the BisqueRNA algorithm to further validate this finding with large cohorts using single-cell data deconvolution transcriptomes. At the same time, we also found that the ratio of endothelial cells to tumor-associated fibroblasts was also higher in the high PCI group than in the low PCDI group. (see page 15 line 366-369)

Reviewer B

1. Abstract

a) Please extend the content of the Background. This paragraph should contain ‘study background’ and ‘study objective’.

Reply 4a: Thank you for your advices. We have added our ‘study objective’ to the content of the Background.

Changes in the text: we have modified our text as advised (see Page 3, line 39-43).

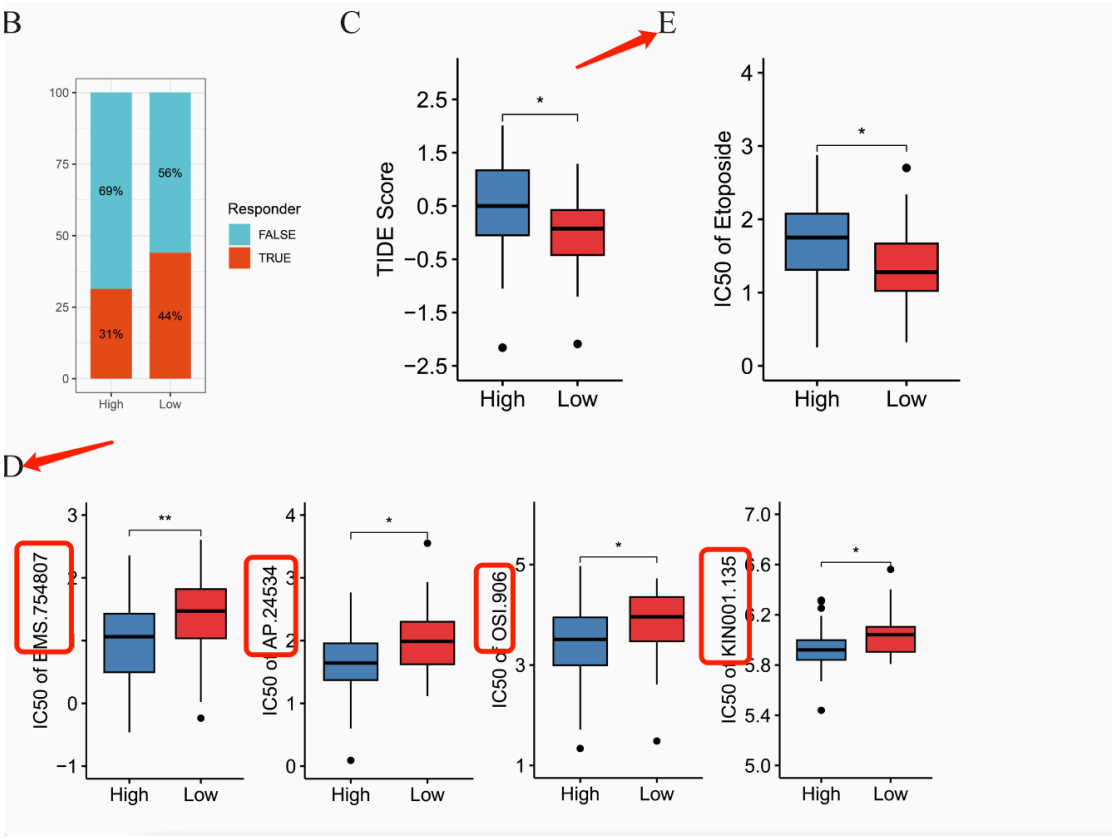
2. Figures

a) Please define all symbols in legend which appeared in the figure. Such as *, **, *** etc.

Reply : Thank you for your careful review. We have added these figure legends (see line 715, 745, 760).

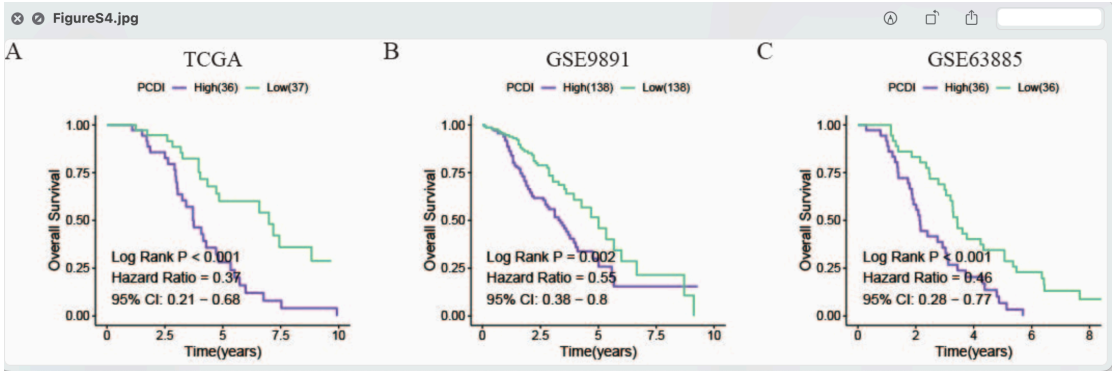
b) Please check the legend, they are not matched.

comparing TIDE score in high- vs. low-PCDI groups. (D) Box plots comparing IC50 of Etoposide, a cell cycle specific antitumor agent, in high- vs. low-PCDI groups. (E) Box plots comparing IC50 of tyrosine kinase inhibitors, including BMS.754807, AP.24534, OSI.906 and KIN001.135 in high- vs. low-PCDI groups.



Reply : Thank you for your careful review. We've corrected it. (See Figure 7 and its figure legend, line 757-760)

c) Figure S4 was not cited, please indicate where to cite. Please also provide a legend for figure S4.

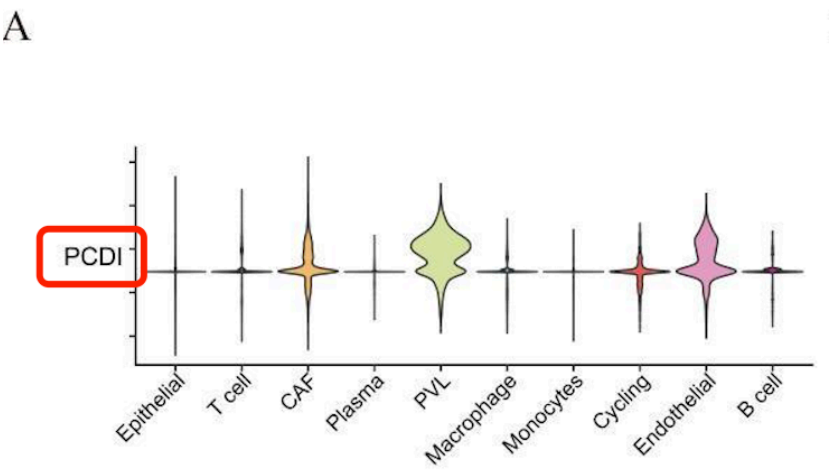


Reply: Thank you for your careful review. We've added it. (See line 786)

3. Figure S3

a) Please check if the figure matches the legend.

Figure S3. (A) The score of PCD for each cell types. (



Reply 3a: Thank you for your careful review. We've corrected it. (See line 773)

b) Please provide the description of S3H in the legend.

Reply 3b: Thank you for your careful review. We've corrected it. (See line 786).

4. Figure 1

As there are no symbols "*, **, ***" in the figure, please delete the explanation in the legend.

Reply: Thank you for your advice. We have deleted the symbols "*, **, ***" in the legend.

Changes in the text: See Line 637.

5. Figure 2

Please provide the meaning of the symbols "*, **, ***" in the legend.

Reply: Thank you for your advice. We have deleted the symbols "*, **, ***" in the legend.

Changes in the text: See Line 646-647

6. Figure 3

Please check the spelling.

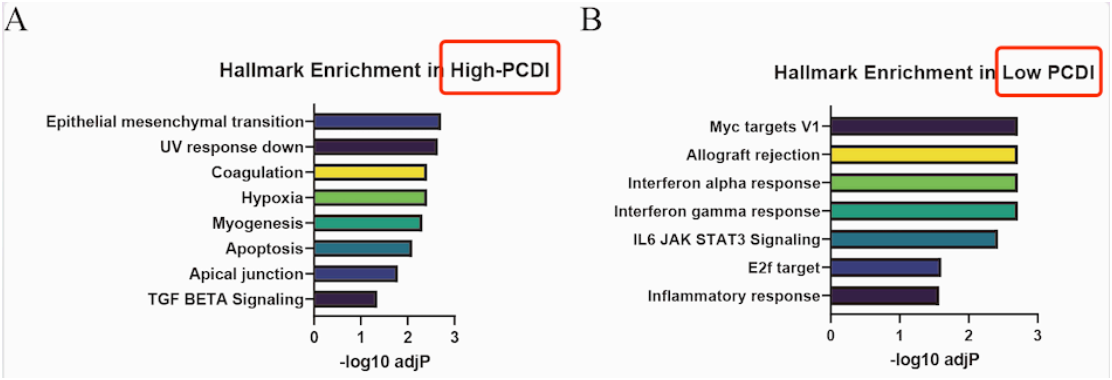
Figure 3. Establishment and evaluation of a programmed cell death index (PCDI). (A) Mutiple Lasso and Cox regression was used to define optimal model. (B) Kaplan-Meier of overall survival

Reply: Thank you for your advice. We have corrected the spelling.
Changes in the text: See Line 652.

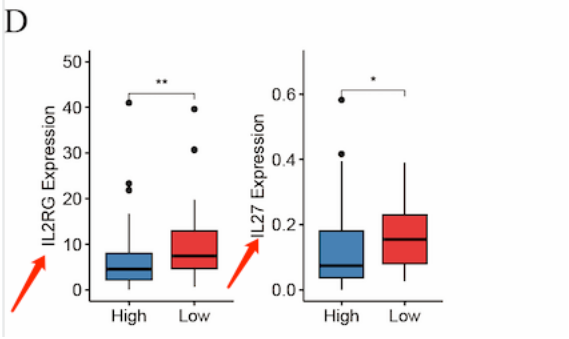
7. Figure 4
Please provide the meaning of the symbol "**" in the legend.
Reply: Thank you for your advice. We have deleted the symbols "***" in the legend.
Changes in the text: See Line 663.

8. Figure 5
a) Please check if the figure matches the legend.

Figure 5. Immune and functional landscapes of low- and high- PCDI groups. (A) GSVA enrichment of HALLMARK pathways in high- and low- PCDI groups. (B) Box plots comparing expression levels of chemokines and receptors in high- vs. low-PCDI groups. (C) Box plots comparing expression



levels of interferons and receptors in high- vs. low-PCDI groups. (D) Box plots comparing expression levels of interferon XCL1 in high- vs. low-PCDI groups. (E) Box plots comparing single-sample gene



Reply 8a. Thank you for your advice. We have completed the modification of Figure 5 and the legend.

Changes in the text: See Line 666-672. See Figure 5.

b) Please also provide the description of figure 5F in the legend. And please check if the desecription of 5E matched with the figure.

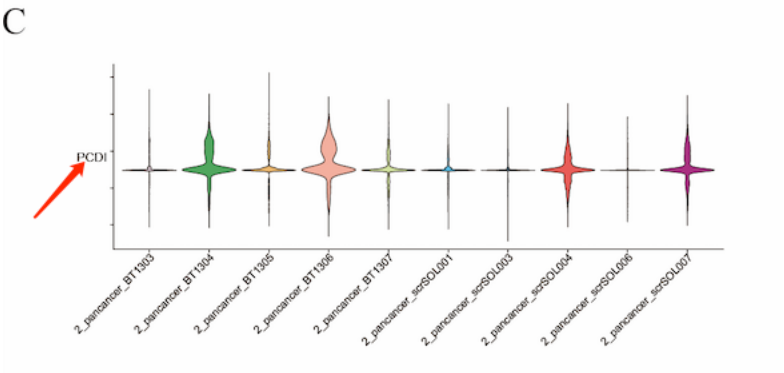
Reply 8b. Thank you for your advice. We have completed the modification of Figure 5 and the legend.

Changes in the text: See Line 666-672.

9. Figure 6

Please check if the figure matches the legend.

777 markers for each of the ten cell types. (C) The score of PCD for each OV samples. (D) Box plots

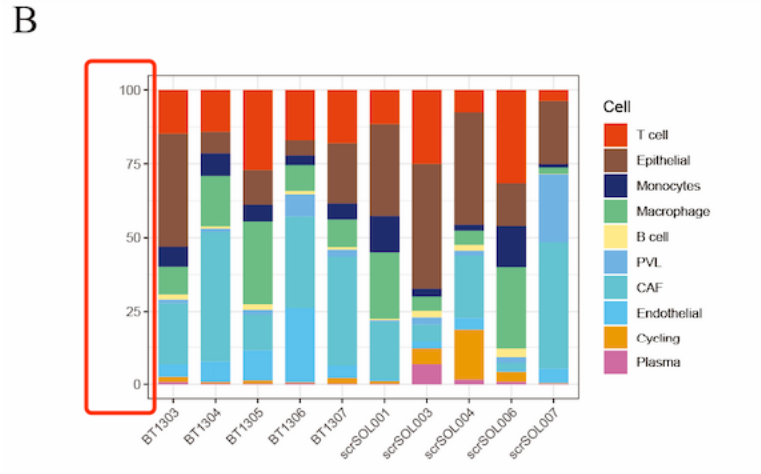


Reply 9. Thank you for your advice. We have completed the modification of Figure 6 and the legend.

Changes in the text: See Line 680-682. See Figure 6.

10. Figure S3

Please provide the unit of the y-axis if applicable.



Reply 10: Thank you for your advice. We have completed the modification of Figure S3. See Figure S3.