Peer Review File

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Reviewer A

The study examines the impact of the co-mutation of TP53 and FLG2 in patients with serous ovarian cancer. The authors obtained the dataset from the cBioPortal and assessed how the co-mutation affects prognosis and immune response. The study found that patients with the co-mutation had longer OS, PFS, and DSS. Additionally, this group showed a higher tumor mutation burden. Transcriptomic analysis revealed increased expression of immune system-related genes in the co-mutation patient group, suggesting an induced immune activation.

Abstract

1. The authors state that the co-mutation group exhibits both higher TMB and MSI. However, in the full cohort of 585 patients, only TMB was significantly elevated, while there was no significant difference in MSI. The significant difference in MSI was observed only in the subgroup of 300 patients. To avoid misrepresentation, the abstract should focus solely on the significant associations found in the entire patient cohort.

Reply: Thank you for your professional advice. In the TMB and MSI analysis of 585 ovarian cancer patients, the co-mutation group had higher scores whether the samples were divided into four or two groups in the TMB analysis. In the MSI analysis, we found that only in the four groups of samples, the comutant group was only higher than the wild-type (FLG2-/TP53-) samples.To avoid misrepresentation, we have modified this part (see page2 line37-40).

Introduction

2. Line 49-50: The phrase "most of the several previous studies" is misleading, as only one study is cited. If there are indeed multiple studies, they should be referenced here. If not, this should be rephrased to accurately reflect the literature. Are there additional studies besides the one cited that report FLG2 as a predictive marker for ovarian cancer prognosis?

Reply: We appreciate your professional comments on our article. At present, there are very few studies on FLG2 gene mutation in ovarian cancer, and the reference we quoted only made a KM survival curve to briefly look at the correlation between FLG2 gene mutation and survival prognosis of ovarian cancer patients. We have revised this part of the narrative (see page 4 line 73-75).

3. Line 52-53: The statement "In previous studies, it has been reported that the single gene mutations of TP53 and FLG2 have predictive value for the prognosis of ovarian cancer" lacks a citation. This needs to be properly referenced, especially since it's a critical point for the study’s aims.

Reply: We are very grateful to the experts for this opinion, and we have added references to this part (see page4 line77).

4. The rationale for evaluating the co-mutation of TP53 and FLG2 as a prognostic marker is not clearly articulated. Since the predictive value of each mutation individually has been demonstrated, what is the reasoning behind exploring their co-mutation? Also, FLG2 is only the fifth most commonly mutated gene in ovarian cancer (as shown in Figure 1A), so why was it specifically chosen for co-mutation analysis?
Reply: Thanks to the reviewers' valuable comments. We added the elaboration of this part (see page 4 line77-79).

5. Additionally, the authors should propose a hypothesis regarding why the co-mutation of specifically these two genes might be relevant for treatment selection in immunotherapy, particularly given that FLG2 does not currently have an established connection to immunotherapy.
Reply: As the review experts said, there are few studies on FLG2 gene mutation and immunotherapy, so we tried to explain the relationship between co-mutation and immunotherapy from the perspective of immune microenvironment (see page4 line83-85).

Results and Discussion

6. Lines 126-127: The percentages of patients in the four groups should be explicitly stated. How many patients have a co-mutation? This is crucial for understanding the distribution and significance of the findings.
Reply: We annotated the number of patients in these four groups (see page 8 line 159-164).
7. Survival Curves: All survival curves presented in the paper should follow the traditional step plot format. The current format should be adjusted accordingly
Reply: We have modified all survival curves.
8. Line 131: Clarify that the p-value here corresponds to Figure 2A
Reply: We have confirmed that the p value is consistent with the figure (see page8 line167).

9. Lines 132, 133, 135, 137, 140/141: Add the corresponding p-values in each instance where significance is discussed.
Reply: We have added the p-values in each instance (see page 8-9 line155-19).
10.Line 137: "Analyzed" should be changed to "analyze"
Reply: we have modified it (see page8 line173).
11. Do the individual mutations of TP53 and FLG2 show significant associations with OS, PFS, and DSS?

Reply: Thanks very much for the professional opinions of the reviewers, we have added the data about the associations between individual mutations of TP53 and FLG2 with prognosis (see page 8 line154-159).

12. The manuscript should clarify whether the significant associations found are specific to the co-mutation of these two genes or if either mutation on its own also shows a significant association with OS, PFS, and DSS. In Lines 230-231, it is stated that no significant difference in prognosis was found between TP53 single mutation and the wild-type, but this data is not shown in the manuscript. It would be helpful to present this analysis to substantiate the claims made.
Reply: we have added the data about the associations between individual mutations of TP53 and FLG2 with prognosis (see page 8 line 154-159).
13. Figure 3A: This figure suggests that the majority of the TMB comes from the TP53 mutation, while the contribution of FLG2 appears non-significant. Have the authors directly compared the TMB between TP53-mutated and FLG2-mutated groups? This would strengthen the conclusions regarding the relative impact of each mutation on TMB.
Reply: We have increased the TMB analysis data in the TP53 mutant group and the FLG2 mutant group (see page 9 line 181-183).

13. Figure 3E and Lines 156-158: The conclusion stated in Lines 156-158 appears somewhat misleading. Only patients with a co-mutation and high TMB score have a better prognosis, while those with a low TMB score have a worse prognosis. This nuance should be made clear in the discussion. Additionally, have the authors investigated whether stratifying patients in the NCM group (no co-mutation) by high and low TMB scores also results in a significant association with survival? Furthermore, has the analysis been performed for all patients, stratified solely by TMB? This could provide additional insights into the role of TMB in survival across all groups.
Reply: We added this part of the data (see page 9 line187-193).
15. Figure 5E-G: These figures should be moved to the Supplementary Information section, as they largely verify the findings already presented in Figure 3 (for all 585 patients) within the smaller subset of 300 patients with RNASeq data. The redundancy of this data detracts from the novelty of the results.
Reply: We have placed these figures in supplemental Figure 2.
16. Lines 199-201: The conclusion here is redundant, as the findings were already presented in Lines 154-158. Moreover, the MSI difference is not significant in the larger group of 585 patients, only in the subgroup of 300. This distinction should be made more clearly to avoid overgeneralization.
Reply: We have removed this part of the content (see page 10).
17. Line 192: While the authors report differences in the expression of immune system-related genes, no significant difference was found in the commonly used immune checkpoint genes. This lack of difference should be discussed in more detail, as it is critical to the relevance of these findings for immunotherapy.

Reply: Common immune checkpoints such as PD1 and PD-L1 in co-mutated patients did not change significantly, while immunotherapy indicators such as TMB were significantly elevated, which called into question traditional immunotherapy targets (see page16 line334-342).

18.The KEGG analysis reveals three pathways—thermogenesis, Parkinson's disease, and oxidative phosphorylation—but there is no clear discussion on why these particular pathways might be deregulated in patients with a co-mutation of TP53 and FLG2.

Reply: We have added a detailed discussion in the revised manuscript regarding the potential implications of the deregulation of the thermogenesis, Parkinson's disease, and oxidative phosphorylation pathways in the context of ovarian patients with TP53 and FLG2 co-mutations (see page14 line299-307).

19. The authors found an upregulation of M2 macrophages in the Co-mutation group (Figure 5C) and state in the discussion that these are significantly associated with prognosis (Line 264/265). It should be explicitly stated that M2 macrophages are typically associated with poor prognosis, as they promote immune evasion. Additionally, the authors suggest that the co-mutation might promote the infiltration of M2 macrophages, but this seems contradictory given the finding of better prognosis in these patients. The authors should provide an explanation for this apparent contradiction.

Reply: we have modified the diassution of M2 in the co-mutation group (see page 15-16 line325-334).

20. A major limitation of the study is that the authors do not show whether the effects observed are also present in patients with either a TP53 or FLG2 mutation alone. Demonstrating the necessity of the co-mutation group is critical for establishing the relevance of the findings.

Reply: We appreciate this insightful comment. In our study, we have analyzed the effects in patients with TP53 and FLG2 mutations separately, and we have included the results in the revised manuscript. The data indicate that the co-mutation group exhibits distinct effects that are not observed in the single mutation groups. This information has been added to the results section to clarify the relevance of the co-mutation findings.

Reviewer B

1. Abstract
    1) Please unify the abbreviations of PD.

In the abstract: 51 Parkinson's disease (PD),

In the text: 226 parkinson disease (PD), and

Reply: we have modified the abbreviations of PD (see page11 line233).

    2) The number of keywords should not exceed 5. Please check and revise.

Reply: we have modified it (see page3 line58-59).

2. Main text

1) Please also indicate the URL for TCGA.

{Ovarian Serous Cystadenocarcinoma, [The Cancer Genome Atlas (TCGA), PanCancer Atlas] }(https://www.cbioportal.org/) database and the samples with

Reply: We have indicated URL for TCGA (see page5 line104).

2) A subheading "Statistical analysis" description in the Methods section must be provided for an Original Article. Please add.

Reply: We have added "Statistical analysis" description(see page 7-8line145-150).

3) Please unify the full name of DEG.

212 **Differential genes (DEGs) analysis between the group of co-mutation and the**

109 **Differentially expressed genes (DEGs) analysis**

Reply: We have unified the full name of DEG.

4) Please check the cohort number.

132 This nomogram was built to evaluate the prognostic significance of these features in a

133 cohort of 571 samples. The overall performance of the model was assessed, yielding a

90 The clinical data of 585 OV samples, including tumor mutation burden (TMB)

91 microsatellite instability (MSI), and mutations data, were retrieved from the cBioPortal

Reply:585 is the total sample, and 571 is the number of samples with prognostic information, which is also modified in the paper(see page7 line141).

5) The Main text should be structured with Introduction, Methods, Results, Discussion, and **Conclusions**. Please modify your article to it.

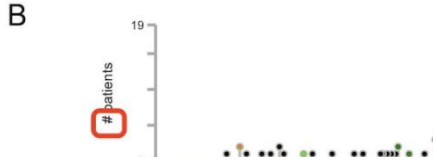
Reply: we have modified it (see page18 line378-382).

6) Please check all abbreviations in the main text, such as FC, AUC, etc. All abbreviated terms should be full when they first appear.

Reply: we have indicated the abbreviation.

3. Figures

1) Figure 1B-C: please indicate the meaning of # in the legend.



Reply: We have indicated it in the legend.

2) Figure 1B-C: please provide description for the x-axis.

Reply: We have added description for the x-axis.

3) Figure 2: please unify the full name of PFS.

531 overall survival; PFS, progress free survival; DSS, disease specific survival; NCM,

Reply: We have unified the full name of PFS.

4) Figure 2: Please check the citation of Figure 2 in your main text. Figure 2F appears earlier than Figure 2E in your text, which is not allowed.

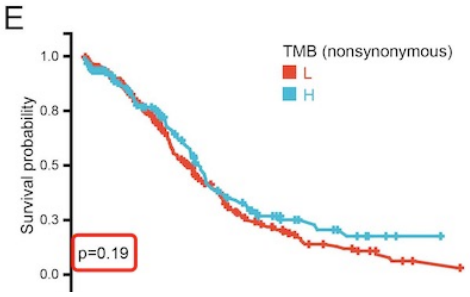
168 had longer OS (p=0.04) and DSS (p=0.02)(Figure 2D, 2F). However, no significant

169 differences were observed in PFS (p=0.25) (Figure 2E).The 585 samples of OV,

Reply: we have modified it.

5) Figure 3E: please unify the data.

201 difference in high and low TMB scores between the two groups (p=0.34; Figure 3E).



Reply: We have modified it.

6) Figure 3: please provide the full name of NCM in the legend.

Reply: We have added the full name of NCM.

7) Figure 3: there is no * and ** in Figure 3, while it is indicated in the legend. Please check.

Reply: We have removed it.

8) Figure 4: please provide the full name of CC, PCA, OS in the legend.

Reply: we have added these content.

9) Figure 5: Please indicate the meaning of – in the legend.

Reply: We have modified it.

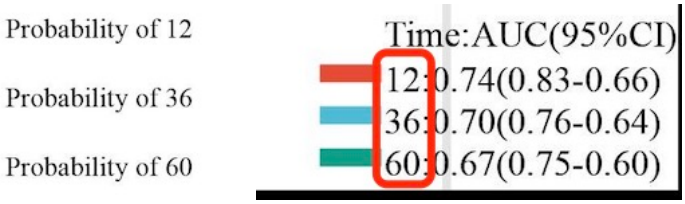
10) Figure 5: there is no “ns” in Figure 5, while it is indicated in the legend. Please check.

Reply: We have removed it.

11) Figure 5: there is no TMB, MSI, ns in the figure. Please remove it from the legend.

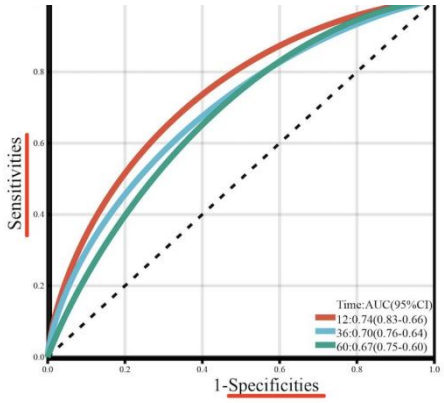
Reply: We have removed it.

12) Figure 6A, 6D: please revise “12, 36, 60” to “1-, 3-, 5-year”.



Reply: We have modified it.

13) Figure 6D: please revise "1-Specificities" to "1-Specificity" and "Sensitivities" to "Sensitivity".



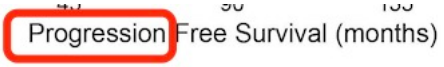
Reply: We have modified it.

14) Figure 6: please provide the full name of AUC, CI, TMB in the legend.
Reply: We have provided the full name of AUC, CI, TMB in the legend

15) Figure S1: please provide the full name of HR, CI, NCM in the legend.
Reply: We have provided the full name of HR, CI, NCM in the legend.

16) Figure S1: please unify the full name of PFS.

568 (A-C). OS, overall survival; PFS, progress free survival; DSS, disease specific survival.



Reply: We have modified it.

17) Figure S2: please indicate the meaning of *, ****, ns in the legend.
Reply: We have indicated the meaning of *, ****, ns in the legend.

18) Figure S1: please provide the full name of NCM in the legend.
Reply: We have modified it.

4. Table 1

1) Please provide the full name of OV, CI, MSI, TMB in the table footnote.

Reply: We have provided the full name of OV, CI, MSI, TMB in the table footnote.