nature portfolio

Peer Review File

Neoadjuvant pembrolizumab in stage IV high-grade serous ovarian cancer: the phase II Neo-Pembro trial

Corresponding Author: Ms S. Lot Aronson

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I would like to thank authors for their revised version and thorough response to my comments as prior reviewer. Overall most of my comments have been addressed in the revised version of the manuscript.
I have a few minor comments to add:
-Retrospective comparison with the historical cohort: Consider removing this from the abstract section. The way the historical cohort was selected is not described in the abstract, and it looks out of place. In my opinion it is a biased comparison and needs further contextualization. Consider just keeping it in the text.
-134: historical cohort. Consider describing in the text (not just supplementary table) the similar utilization of front-line PARPi in both cohorts. I think this is a question that will be raised by readers.
-143: You report that PFS was statistically different between the study and historical cohort. Yet the CI crosses 1, and p is non significant (HR 0.31 [95%CI 0.08-1.25], p=0.098). Could you comment on it or readdress it?
-HRd: number of large scale state transitions were assessed. How does this technique correlate with the currently approved HRd measures in ovarian cancer? If different or not validated, consider discussing it as a limitation of your study
-Minor typo: (647) “mentioned above.. Samples were then classified” – Typo with double period.

Reviewer #3

(Remarks to the Author)
Immune checkpoint inhibitors (ICIs) generally have limited efficacy in high-grade serous ovarian cancer (HGSOC), although some patients experience lasting responses. The phase 2 Neo-Pembro study (NCT03126812) evaluated pembrolizumab (pembro) following one cycle of chemotherapy in 33 stage IV HGSOC patients. Findings revealed immune activation markers (increased CD3+, CD8+, CD8+/FOXP3+ ratio, TNF-α, and interferon-γ) and major pathologic responses in 27% of patients. Major responders showed prolonged progression-free survival (PFS) and overall survival (OS), with ctDNA clearance emerging as a promising indicator. PD-L1 expression and homologous recombination deficiency (HRD) were associated with response, suggesting potential biomarkers. Major responders treated with pembro showed improved OS compared to a historical cohort, suggesting long-term survival benefits with neo-adjuvant pembro.

The authors have made efforts to address prior review comments, but some key points remain inadequately addressed, impacting the conclusiveness of the findings.

1. While the authors acknowledge potential biases from using historical controls, further discussion on how this approach and possible confounding factors could affect key outcomes (OS and PFS) would strengthen the manuscript, especially when the sample size is very small.
2. The manuscript notes challenges in isolating pembrolizumab’s effects due to the lack of a control arm. However, it lacks an in-depth examination of neoadjuvant chemotherapy’s potential role in immune activation.
3. PD-L1 expression is mentioned as a potential biomarker, but the ATALANTE and ANITA trials, which question PD-L1’s efficacy as a biomarker in ovarian cancer, are only briefly referenced. A more comprehensive discussion on why PD-L1 may have shown limited utility in other trials (e.g., methodological differences, variations in patient populations) would be valuable. Including a table or supplementary figure summarizing recent PD-L1 findings in ovarian cancer could also enhance the discussion.

4. Although the authors explain the exclusion of PARPi and bevacizumab in frontline therapy due to local guidelines, questions remain about the broader applicability of the study's findings across different treatment settings.
5. While ctDNA clearance is associated with response, its clinical significance and potential as a real-time monitoring tool are not fully explored. Further discussion on how ctDNA could guide treatment adjustments in practice would add depth to the findings.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Dear Dr Aronson,

Thank you for the opportunity to review the third version of the manuscript. I believe that the quality of the manuscript has significantly improved and all my comments have been addressed.

Best regards,

Ainhoa

Reviewer #3

(Remarks to the Author)
Thank you to the authors for the effort in addressing all the comments. I have no further comments.

Reviewer #1

I would like to thank authors for their revised version and thorough response to my comments as prior reviewer. Overall most of my comments have been addressed in the revised version of the manuscript.

I have a few minor comments to add:

-Retrospective comparison with the historical cohort: Consider removing this from the abstract section. The way the historical cohort was selected is not described in the abstract, and it looks out of place. In my opinion it is a biased comparison and needs further contextualization. Consider just keeping it in the text.

Reply:

We thank the reviewer for the valuable feedback. In line with this suggestion, we have removed the retrospective comparison with the historical cohort from the abstract.

-134: historical cohort. Consider describing in the text (not just supplementary table) the similar utilization of front-line PARPi in both cohorts. I think this is a question that will be raised by readers.

Reply:

We have added the following description on the use of front-line PARPi in both cohorts to the main body text on lines 138-139: "*The use of PARP inhibitors as front-line maintenance treatment was similar in the historical and intervention cohort (11.5% vs. 9.1% [p=0.626], respectively).*"

-143: You report that PFS was statistically different between the study and historical cohort. Yet the CI crosses 1, and p is non significant (HR 0.31 [95%CI 0.08-1.25], p=0.098). Could you comment on it or readdress it?

Reply:

Thank you for pointing this out. We confirm that the reported numbers are correct (HR 0.31 [95% CI 0.08–1.25], p=0.098), and have corrected the original text in lines 144-145: "*Notably, OS of major responders in the study cohort was significantly better than OS of major responders in the historical cohort (Fig. 1c, median OS NR [95%CI NR-NR]) vs 30.7 months [95%CI 24.6-NR], HR 0.12 [95%CI 0.01-0.97], p=0.047),* ***while PFS was not*** *(median PFS NR [95%CI 30.3-NR] vs 24.6 months [95%CI 12.9-NR], HR 0.31 [95%CI 0.08-1.25], p=0.098)."*

-HRD: number of large scale state transitions were assessed. How does this technique correlate with the currently approved HRD measures in ovarian cancer? If different or not validated, consider discussing it as a limitation of your study

Reply:

Thank you for your comment. The large-scale state transition (LST) score used to assess HRD status in our study has shown a strong correlation with FDA-approved HRD measures, including the Myriad MyChoice HRD test, as demonstrated in previous studies (Sztupinszki et al., 2021). Furthermore, the LST score has been suggested to be slightly superior in some contexts (Christinat, 2023). However, it is important to note that the LST score is based on genomic scarring, which may not always reflect the functional HRD status of tumors, potentially leading to incorrect classifications in certain cases. We have added this consideration as a limitation of our study in the revised manuscript to provide clarity (lines 394-397). Specifically, we now state that while the LST score strongly correlates with FDA-approved HRD tests, it can still misclassify HRD status in some tumors, particularly since it assesses genomic scarring rather than functional HRD status.

-Minor typo: (647) "mentioned above.. Samples were then classified" – Typo with double period.

Reply: We have resolved this typo.

Reviewer #3

Immune checkpoint inhibitors (ICIs) generally have limited efficacy in high-grade serous ovarian cancer (HGSOC), although some patients experience lasting responses. The phase 2 Neo-Pembro study (NCT03126812) evaluated pembrolizumab (pembro) following one cycle of chemotherapy in 33 stage IV HGSOC patients. Findings revealed immune activation markers (increased CD3+, CD8+, CD8+/FOXP3+ ratio, TNF-α, and interferon-γ) and major pathologic responses in 27% of patients. Major responders showed prolonged progression-free survival (PFS) and overall survival (OS), with ctDNA clearance emerging as a promising indicator. PD-L1 expression and homologous recombination deficiency (HRD) were associated with response, suggesting potential biomarkers. Major responders treated with pembro showed improved OS compared to a historical cohort, suggesting long-term survival benefits with neo-adjuvant pembro.

The authors have made efforts to address prior review comments, but some key points remain inadequately addressed, impacting the conclusiveness of the findings.

1. While the authors acknowledge potential biases from using historical controls, further discussion on how this approach and possible confounding factors could affect key outcomes (OS and PFS) would strengthen the manuscript, especially when the sample size is very small.

Reply:
Thank you for raising this important point. We have expanded the discussion in the manuscript to address how differences between the cohorts could potentially influence PFS and OS results. For further contextualization, we highlight the importance of considering residual confounding as a potential source of bias when interpreting the comparison with the historic cohort (lines 284-291). Specifically, patients in the historical cohort were older than those in the interventional cohort (median age: 69 vs. 64 years), which may contribute to worse survival outcomes in the historical group. Conversely, the shorter median interval between neo-adjuvant therapy and surgery in the historical cohort (31 vs. 38 days) is a known prognostic factor associated with improved outcomes, potentially favoring the historical cohort in survival analyses.

2. The manuscript notes challenges in isolating pembrolizumab's effects due to the lack of a control arm. However, it lacks an in-depth examination of neoadjuvant chemotherapy's potential role in immune activation.

Reply:
Thank you for your comment. To further describe the role of neoadjuvant chemotherapy in immune activation, we included its effect on increased T-cell densities, upregulation of MHC class I expression, and modulation of the T cell-to-regulatory T cell balance. Additionally, chemotherapy induces PD-L1 overexpression, which typically inhibits immune responses. However, in the context of immune checkpoint inhibition, elevated PD-L1 can sensitize patients to pembrolizumab, potentially improving disease control (lines 300-303),

3. PD-L1 expression is mentioned as a potential biomarker, but the ATALANTE and ANITA trials, which question PD-L1's efficacy as a biomarker in ovarian cancer, are only briefly referenced. A more comprehensive discussion on why PD-L1 may have shown limited utility in other trials (e.g., methodological differences, variations in patient populations) would be valuable. Including a table

or supplementary figure summarizing recent PD-L1 findings in ovarian cancer could also enhance the discussion.

Reply:
We appreciate the suggestion to provide a more comprehensive discussion of the limitations of PD-L1 as a biomarker in ovarian cancer, particularly in light of the ATALANTE and ANITA trials. To address this, we have expanded the discussion to highlight key factors that could contribute to the divergent results observed across trials (lines 321-332). We specifically mention how the ATALANTE and ANITA trials used a ≥1% immune cell positivity threshold for PD-L1, which may not be the most optimal cutoff in ovarian cancer, and how the exclusion of tumor cell expression in these studies could further impact predictive value. Furthermore, variation in assays and scoring methods across studies further complicates interpretation and highlights the need for standardization. We feel that a table summarizing the recent PDL-1 finding as a biomarker falls outside the scope of this manuscript, but will be happy to reconsider if the editor believes this to be a valuable addition.

4. Although the authors explain the exclusion of PARPi and bevacizumab in frontline therapy due to local guidelines, questions remain about the broader applicability of the study's findings across different treatment settings.

Reply:
Thank you for your comment regarding the broader applicability of our findings. Extrapolating our results to settings with more widespread use of PARPi or first-line bevacizumab therapy requires consideration of the potential interactions between ICIs, PARPi, and bevacizumab, both in terms of efficacy and safety. We have further discussed these potential interactions and referenced relevant trials conducted in other treatment settings (lines 342-363). Ongoing trials are currently evaluating the efficacy of the triplet combination in the frontline setting, and once results are available, they may provide further insights into the applicability of our findings to broader clinical contexts.

5. While ctDNA clearance is associated with response, its clinical significance and potential as a real-time monitoring tool are not fully explored. Further discussion on how ctDNA could guide treatment adjustments in practice would add depth to the findings.

Reply:
Thank you for your comment. We agree that the clinical utility of ctDNA dynamics warrants further exploration. We have extended the discussion of how ctDNA could guide treatment adjustments in practice, if further validated (lines 375-382). These data suggest that monitoring ctDNA clearance may provide valuable insights into treatment response and prognosis, with the potential to guide clinical decisions. For instance, if ctDNA clearance is not achieved after three cycles of neoadjuvant chemotherapy, extending treatment to six cycles may be considered. Alternatively, non-responding patients might benefit from an early transition to a non-cross-resistant regimen to enhance therapeutic efficacy. Possibly, in cases where ctDNA clearance is not achieved, the contribution of cytoreductive surgery to improve outcomes becomes uncertain and might be challenged. Such strategies warrant further exploration in larger, randomized studies and may ultimately help to optimize patient management.