Peer Review File

Reviewer A

Comment 1:

The article is well-written and effectively describes the undertaken research. The authors state their objective as follows: "In this retrospective study, we aimed to uncover the association between HER2 status, specifically HER2-low and HER2-zero, and the prognosis of HR-positive breast cancer."

However, it's essential to note that the study cohort, as acknowledged by the authors in the limitations section, is not only retrospective but also small and unrepresentative. The authors assert no survival difference between luminal A and luminal B, despite well-established knowledge of such differences in survival between these two subsets of breast cancer.

The reported percentage of HER2-low versus HER2-zero concerning luminal A and B is nearly equal, which could be valuable information if the study had an adequate number of cases. Unfortunately, the data are insufficient for meaningful conclusions.

Furthermore, the abstract and the overall manuscript suggest that the analysis aims are to determine whether patients with HER2-low breast cancer could benefit more from a new anti-HER2 drug, Trastuzumab deruxtecan (T-DXd), compared to HER2-zero. T-DXd is a conjugate containing an antibody-drug (trastuzumab) with a camptothecin derivative possessing topoisomerase I inhibitor activity. This new conjugaten has been reported to perform better, than traditional anti-HER2 treatments and in HER-2 low cases. The topoisomerase inhibitor component could suggest potential efficacy in luminal B, which is known to be more proliferative than luminal A.

In conclusion, the study needs to be replicated with a more representative cohort, and the study's objective should be clarified. The current findings are limited by the small and unrepresentative nature of the cohort, and the intended focus on the efficacy of T-DXd in HER2-low breast cancer patients would benefit from a more robust and clearer research design.

Reply 1:

Thank you for reviewing our manuscript and providing insightful comments and suggestions that have helped us to significantly improve the quality of the manuscript.

According to your suggestion, we collected additional cases from January 2018 to December 2018 as our retrospective study had a small number of case and unrepresentative nature.

We wanted to investigate whether the HER2 status (HER2-low and HER2-zero) has a prognostic impact on ER-positive breast cancer, especially luminal A-like and luminal B-like, in a real-world clinical setting. However, this study did not directly compare luminal A-like with luminal B-like, but aimed to investigate the association between HER2-low and HER2-zero of each luminal type. Moreover, these survival analyses suggested that OS was significantly different in HER2-zero luminal A-like and RFS was shorter in HER2-low luminal B-like breast cancer.

After further treatment of HER-low breast cancer with T-DXd, we aim to investigate the efficacy of T-DXd on them.

Changes in the text:
We have modified the text, as advised (see Page 3, line 38-47; Page 7, line 103-105; Page 10-13, line 157-229).

Reviewer B

Summary:
This retrospective study compares clinic-pathological characteristics and survival in ER-positive breast cancer patients with HER2-low and HER2-zero status, further analyzing their prognostic value in luminal A-like and luminal B-like subtypes. While the study finds no impact of HER2 low/zero on RFS and OS in all included patients, a potential difference in the prognosis of HER2 low/zero in luminal A- and B-like breast cancers is noted.
Strengths: The research subject is interesting with sound data.
Weaknesses: issues with statistical analysis, flow, and existing similar research.

Major Comments:
1. Statistical Analysis:
Usually, chi-square tests and t-tests are used to test differences between two groups, while univariate, multivariate, and logistic regression analyses are used to test associations. Therefore, if the "univariate P-value" in the results represented the P value on differences of variables between the HER2-low and HER2-zero groups, then multivariable analysis on that is not appropriate.
Instead, suggest adding univariate and multivariate analyses in survival to identify prognosis predictors and hazard ratios for RFS and OS.

Reply 1:
Thank you for your comments and suggestions.
We primarily revised the univariate analysis in survival to identify prognosis predictors and hazard ratios for RFS and OS, according to your suggestion. However, Cox regression analysis is performed only when survival analysis revealed a proportional hazard; we performed univariate analysis for OS in luminal A-like breast cancer and RFS in luminal B-like breast cancer subgroup.

Changes in the text:
We have modified the text, as advised (see Page 9-10 line 145-146, 150-153; Page 12, line 209-215; Page 13, line 226-229).

2. Introduction:
Suggest including background knowledge and a literature review on HER2-negative/low in the

"rationale and knowledge gap" to provide context and enhance logical flow.

Reply 2:
Thank you for your comment. We have searched the journals with background knowledge and a literature review on HER2-negative/low breast cancer, and added the text accordingly.

Changes in the text:
We have modified our text, as advised (see Page 7 line 87-93).

3. Results:
In Part 2.1, suggest that the description of patient characteristics be more descriptive, collative, and concise.

Reply 3:
Thank you for your suggestion. We have made baseline characteristic variables concise.

Changes in the text:
We have modified the text, as advised (see Page 7-9 line 103-140; Page 10-12, line 157-196; Table1).

4. Discussion:
- Suggest adding a literature review and discussion on "HER2-low and HER2-zero may affect prognosis in certain populations," especially in luminal A-/B- like types in Part 4.3.
- Include literature, if available, in Part 4.4 to support explanations.
- Suggest adding clinical meaning of the research findings to connect it with the following discussion on T-DXd in Part 4.5.

Reply 4:
Thank you for your suggestion.
We conducted a literature search on luminal A-like or B-like breast cancer, but no reports were found. However, we discussed the factors of Ki-67, HR, and genetic testing that determine luminal A- or B-like (see Page 15, line 264-267, 271-277).
We referred the article in Part 4.4 to provide further explanation (see Page 16, line 283-284).
We added clinical significance of the research findings in Part 4.5 (see Page 16, line 301-303).

Changes in the text:
We have modified the text, as advised (see Page 15, line 264-267, 271-277; Page 16, line 283-284; Page 16, line 301-303).

Minor Comments:
1.Introduction:

- The definition of HER2-zero breast cancer is lacking.
- Suggest mentioning the clinical significance of the study in the objectives.
- Line 80-81: "It is effective against HER2-positive breast cancer, which displays resistance to conventional anti-HER2 therapy." This sentence needs to be checked.

Reply 1:
Thank you for your comments.
We have added the definition of HER2-zero breast cancer on Page 8, line 109-110.
We have also mentioned the clinical significance of the study in the objectives on page 7, line 97-99.
We have revised the sentence as per your suggestion (Page 6, line79).

Changes in the text:
We have modified the text, as advised (see Page 6, line 79; Page 7, line 97-99; Page 8, line 109-110).

2. Results:
- Line 156 "an average age of" should be corrected as the following numbers are median, not mean.
- "IQR" should be added in the brackets before the number
- Line 232-234: "No difference was observed in the RFS and OS based on luminal types." The description can be more accurate, as the difference in the RFS and OS is between HER2-low and HER2-zero subgroups, not luminal types.

Reply 2:
Thank you for your comments.
We revised "average" to "median".
We added "IQR" in the brackets before the number.
We have also collected additional data and changed the results of survival analyses, and mentioned RSF and OS for HER2-low and HER2-zero each luminal subgroup.

Changes in the text:
We have modified the text, as advised (see Page 10, line 160; Page 10-11, Part 3.1; Page 13, line 226-229).

3. Discussion:
- In Part 4.1, suggest adding a summary of the patients included.
- The limitation of the sample's single-center representation and limited sample size should be mentioned.
- Line 247-249. The "consistency of HER2 evaluations was uncertain" was not clear to me. Even though the ASCO/CAP guidelines did not clearly define the HER2-low status, in this study, there is a clear definition given in the method about HER2-low and HER2-zero.

- Line 276-281 The transition of focus from HER2 low/zero prognostic meaning to different histological characteristics of HER2-low and HER2-zero is abrupt. Suggest having some discussion or transition between.

- The subtitles for parts 4.3 and 4.4 are not accurate. The topic discussed in 4.3, the association of HER2 low/zero and prognosis, is also part of the findings of this manuscript (even though it is a negative result). Also, related literature reviews should be included in 4.4 as well. The authors can probably consider combining 4.3 and 4.4 together.

4.In Figure2, the picture C and D don't appear in the documents for review.

Reply 3:

Thank you for your comment. We added a summary of the patients in Part 4.1.

We have mentioned the limitation, as suggested.

We deleted the sentence "the consistency of HER2 evaluations was uncertain. The ASCO/CAP guidelines facilitated breast cancer classification according to the HER2 status but did not clearly define the HER2-low status (7).".

We have added a discussion in part 4.4.

We combined 4.3 and 4.4 together, as suggested.

We apologize for this and have uploaded Figure2.

Changes in the text:

We have modified the text, as advised (see Page 13, line 233-238; Page 16, line 294-297, 301-303; Part 4.3).