Peer Review File

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Reviewer A

This presents real-world data on patients with HR+ABC who received tucidinostat treatment. Real-world data is crucial for complementing interventional study data. However, RWD generated from a single institution holds lower value than that from a multi-institution.

It is advisable to clarify the study's objectives more explicitly. Did the author focus on assessing the efficacy and safety of tucidinostat based on prior CDK4/6 therapy treatment? If so, the conclusion should be modified (introduction, p4).

The authors should omit the statement 'with particular attention being paid to the initial 6 weeks of treatment' because this is a real-world study reflecting daily practice (method section, p5).

The article should include details on the data source and data collection method. Did the author collect data from a database or electronic medical records? (method section).

The definition of high tumor burden needs to be provided by the author (results section, p7).

The author should explain why the cutoff for ER is set at 50% and PgR at 20% (table 1).

Reply to reviewer A:

Comment 1: Did the author focus on assessing the efficacy and safety of tucidinostat based on prior CDK4/6 therapy treatment? If so, the conclusion should be modified (introduction, p4).
Reply 1: Evaluating the efficacy of tucidinostat in patients previously treated with CDK4/6 inhibitors is only a segment of this study's analysis. Our objective is to identify an optimized treatment approach and determine the patient population that might derive the greatest benefit from tucidinostat. If you have more specific suggestions regarding this matter, please feel free to share them.

Comment 2: The authors should omit the statement 'with particular attention being paid to the initial 6 weeks of treatment' because this is a real-world study reflecting daily practice (method section, p5).
Reply 2: Thank you for your suggestion, we have removed this sentence from the method (p5 line152).
Changes in text: We have modified our text as advised. (page 5 line 152)

Comment 3: The article should include details on the data source and data collection method. Did the author collect data from a database or electronic medical records? (method section).
Reply 3: Thank you for your suggestion. The data sources and data collection methods for this study are described in the Patients and eligibility criteria section (methods section, p5 line 131-

135). Clinical and survival data of patients were derived from Breast Cancer Information Management System and verified in the hospital electronic medical record.
Changes in text: We added the description " Clinical assistants conducted regular follow-ups on these patients through the Breast Cancer Information Management System (BCIMS), regularly documenting patients' clinical and pathological characteristics, diagnoses, treatment courses, efficacy assessments, and dates of death or last follow-up. Each patient's data was verified in our hospital electronic medical records." (page 5 line 131-135)

Comment 4: The definition of high tumor burden needs to be provided by the author (results section, p7).
Reply 4: Thank you for pointing this out. The description of patients with high tumor burden here is ambiguous and should be expressed as patients with visceral metastases with rapid disease progression.
Changes in text: We revised the description "10.64% of the patients with rapidly progressing visceral metastases received tucidinostat plus ET as maintenance treatment." (page3 55-56, page 7 line 204-205)

Comment 5: The author should explain why the cutoff for ER is set at 50% and PgR at 20% (table 1).
Reply 5: Thank you for your suggestion. In clinical practice, ER expression within the range of 1-10% is generally considered low, whereas there isn't a definitive value established for high ER expression. Notably, PR at 20% is considered the threshold distinguishing between Luminal A and Luminal B subtypes. In our study, our aim was to investigate whether varying levels of ER expression affect the efficacy of tucidinostat. Our univariate analysis revealed that patients with ER expression exceeding 50% exhibited better response to tucidinostat treatment compared to those with ER levels below 50%.

Reviewer B

The paper titled "Combination therapy with tucidinostat in patients with advanced hormone receptor-positive human epidermal growth factor receptor 2-negative breast cancer: a real-world study" is interesting. For patients with HR+/HER2-ABC, tucidinostat combination therapy offers certain survival benefits with controllable safety. Furthermore, compared with non-maintenance therapy, maintenance therapy after chemotherapy may have promising efficacy. However, there are several minor issues that if addressed would significantly improve the manuscript.
1) What are the current adjuvant chemotherapy regimens for the treatment of patients with HR+/HER2-ABC? What are the potential next steps? It is recommended to add relevant content to the discussion.
2) Please comprehensively summarize recent advances in tucidinostat as both monotherapy and a regimen of combination therapy in solid malignancies in clinic.
3) Combined with endocrine therapy, what is the impact of molecular subtypes on the prediction of distant recurrence of HR+/HER2-ABC? It is recommended to add relevant

content.
4) How to evaluate the long-term efficacy and safety of tucidinostat in the treatment of HR+/HER2-ABC? It is recommended to add relevant content.
5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Chemotherapy or endocrine therapy, first-line treatment for patients with hormone receptor-positive HER2-negative metastatic breast cancer in China: a real-world study, Ann Transl Med, PMID: 34164465”. It is recommended to quote this article.
6) How to evaluate the role of prognostic factors such as neoadjuvant chemotherapy? What is the impact on the prognosis of women whose pathology has not been completely relieved? It is recommended to add relevant descriptions.
7) The number of patient samples in this study is too small, and a large sample study should be added for verification.

Reply to reviewer B:

Comment 1: What are the current adjuvant chemotherapy regimens for the treatment of patients with HR+/HER2-ABC? What are the potential next steps? It is recommended to add relevant content to the discussion.
Reply 1: Thank you for your advice. In general practice, adjuvant therapy is commonly employed for HR+/HER2- early breast cancer patients post-surgery, with chemotherapy regimen selection often based on anthracyclines or taxanes tailored to the risk of recurrence. However, this study focuses on patients with recurrent/metastatic breast cancer, where potential treatment options include salvage chemotherapy. For patients who have undergone multiple lines of endocrine therapy, single-agent sequential chemotherapy is typically preferred. In cases of rapid disease progression, life-threatening visceral metastases, or the urgent need for rapid symptom control, consideration may be given to combination regimens, with specific drug choices guided by principles applied in triple-negative breast cancer treatment.
Regarding the next treatment option, for patients with germline BRCA1/2 mutations, PARP inhibitors (such as olaparib) are recommended. In cases where HER2 IHC is 1+ or 2+ with ISH negativity, consideration may be given to Fam-trastuzumab deruxtecan in later lines of therapy. Additionally, Sacituzumab govitecan may be considered for later lines. The choice of single-agent or combination chemotherapy remains among the available options.
In this study, more than half (55.81%) of patients chose chemotherapy after the progression of tucidinostat, and 11.63% chose to change to another CDK4/6 inhibitor and endocrine drugs, as detailed in Supplemental materials Table S1.
Changes in text: We add descriptions to the results and discussion section.
After the progression of tucidinostat, more than half (55.81%) of patients chose chemotherapy and 11.63% chose to change to another CDK4/6 inhibitor and endocrine drugs, as detailed in Supplemental materials Table S1. (page 7 line 228-230)

Regarding the next treatment option after the progression of tucidinostat, for patients with germline BRCA1/2 mutations, PARP inhibitors (such as olaparib) are recommended. In cases with low HER2 expression, consideration may be given to trastuzumab deruxtecan in later lines of therapy. Additionally, Sacituzumab govitecan may be considered for later lines. The choice

of single-agent or combination chemotherapy remains among the available options. (page 10 line 309-314)

Comment 2: Please comprehensively summarize recent advances in tucidinostat as both monotherapy and a regimen of combination therapy in solid malignancies in clinic.
Reply 2: Thank you for your suggestion. Tucidinostat was approved for relapsed or refractory (R/R) peripheral T-cell lymphoma, R/R adult T-cell leukemia-lymphoma and ABC, and is the only HDAC inhibitor approved to date for the treatment of solid tumors.
Changes in text: We have added relevant descriptions to the introduction. (page 4 line 93-96)

Comment 3: Combined with endocrine therapy, what is the impact of molecular subtypes on the prediction of distant recurrence of HR+/HER2-ABC? It is recommended to add relevant content.
Reply 3: Thank you for your inquiry. Considering the limitation of a small sample size in our current study, although it did not delve into the relationship between molecular subtypes and distant recurrence, we acknowledge the pivotal value of molecular subtypes in breast cancer treatment and prognosis assessment. We look forward to future research endeavors that delve deeper into exploring the impact of molecular subtypes on predicting distant recurrence, aiming to comprehensively understand the influence of prognostic factors on breast cancer patients' outcomes. If there's any confusion or if you need more information, please don't hesitate to ask again. Your questions are valuable to us, and we're here to provide clear and precise answers.

Comment 4: How to evaluate the long-term efficacy and safety of tucidinostat in the treatment of HR+/HER2-ABC? It is recommended to add relevant content.
Reply 4: Thank you for your inquiry. Evaluating the long-term efficacy and safety of tucidinostat in the treatment of HR+/HER2-ABC is a crucial aspect. While our study focused on the short-term efficacy and safety of tucidinostat, a comprehensive assessment of its long-term effects and potential risks requires the inclusion of a larger patient cohort, longer-term follow-ups, and continuous monitoring. We acknowledge the necessity for these elements and highlight the limitations of our study in the discussion section.
Changes in text: We revised the description " It was a single-center retrospective study with a small sample size and a relatively short follow-up duration. " (page 10 line 316-317)

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Chemotherapy or endocrine therapy, first-line treatment for patients with hormone receptor-positive HER2-negative metastatic breast cancer in China: a real-world study, Ann Transl Med, PMID: 34164465”. It is recommended to quote this article.
Reply 5: Thank you for your suggestion. We cited this article in the introduction section.
Changes in text: We cited this article in the introduction section (page 3 line 74).

Comment 6: How to evaluate the role of prognostic factors such as neoadjuvant chemotherapy? What is the impact on the prognosis of women whose pathology has not been completely relieved? It is recommended to add relevant descriptions.

Reply 6: Thank you for your suggestion. While our study primarily focuses on the application of tucidinostat in ABC, we acknowledge the significance of prognostic factors such as neoadjuvant chemotherapy and achieving postoperative pathological complete response (pCR) in breast cancer treatment. Although our study did not directly explore the impact of neoadjuvant chemotherapy, it is plausible to speculate that women with non-pCR may exhibit resistance to commonly used chemotherapeutic agents such as anthracyclines and taxanes, while potentially retaining sensitivity to endocrine therapies. For these patients, tucidinostat may represent an additional therapeutic strategy that holds promise in improving their prognosis. However, this remains a theoretical assumption, and further comprehensive exploration of these factors' impact on the prognosis of breast cancer patients will necessitate larger sample sizes in future studies.
It is undeniable that the previous treatment does have a complex impact on the efficacy and prognosis of endocrine therapy and targeted therapy for patients with advanced breast cancer, so we added the limitation part.
Changes in text: We added the description " Variations in patients' prior treatments could have influenced the outcomes observed." (page 10 line 314-315)

Comment 7: The number of patient samples in this study is too small, and a large sample study should be added for verification.
Reply 7: Thank you for your valuable suggestion. We are aware of the critical importance of sample size in ensuring the reliability and generalizability of research findings. Despite the relatively small sample size in our current study, we have taken utmost care to ensure the rigor of our data and the reliability of our statistical analyses. While increasing the sample size for further validation is indeed an effective approach, we are currently monitoring recent patients using tucidinostat. However, due to the short follow-up duration, mature results have not yet emerged, preventing us from expanding the sample size at this time.

Reviewer C

1) First, the title needs to indicate efficacy and safety and the clinical research design of this study such as a prospective cohort study. The authors need to consider whether it is appropriate to describe it as a real-world study since real-world studies are often characterized by large samples. In fact, the authors analyzed cases from real-world clinical settings.
2) Second, the abstract needs some revisions. The authors did not describe the clinical needs for real-world data and the limitations of data from RCTs in the background. The methods need to describe the inclusion of subjects, the assessment of efficacy and safety, and follow up procedures. The conclusion needs some comments for the clinical implications of the findings.
3) Third, the authors need to review and analyze the limitations and knowledge gaps of prior studies to support the need for real-world evidence. My major concern is the current data are not from a strictly deigned and retrospective, so the information from this study is not convincing.

4) Fourth, please describe the calculation of sample size of this study and follow up details of this study. In statistics, please indicate the purposes of the Cox regression analysis.
5) Finally, please consider to review and cite several related papers: 1. Yuan Y, Zhang S, Wang T, Wang B, Wang S, Shi J, Sun T, Yin Y, Ouyang Q, Li J, Wen Y, Zhang L, Jiang Z. Efficacy and safety of abemaciclib-based therapy versus tucidinostat-based therapy after progression on palbociclib in patients with HR+HER2− metastatic breast cancer. Transl Breast Cancer Res 2023;4:10.. 2. Neven P, Dullens L, Han S, Deblander A, Van Herck Y, Van Houdt M, Wildiers H. Navigating next-generation HR+/HER2− metastatic breast cancer therapies: a critical commentary on abemaciclib vs. tucidinostat after palbociclib progression. Transl Breast Cancer Res 2023;4:31. 3. Balanchivadze N, Robert NJ. Abemaciclib-based therapy versus tucidinostat-based therapy in patients with HR+HER2− metastatic breast cancer after palbociclib progression: insights and challenges from a comparative cohort study in China. Transl Breast Cancer Res 2023;4:32.. 4. Zhang Q, Li W, Hu X, Sun T, Cui S, Wang S, Ouyang Q, Yin Y, Geng C, Tong Z, Cheng Y, Ning Z, Jiang Z. Tucidinostat plus exemestane for postmenopausal patients with advanced, hormone receptor-positive breast cancer: a long-term safety and overall survival update from the randomised, double-blind, placebo-controlled, phase 3 trial. Transl Breast Cancer Res 2023;4:18.

Reply to reviewer C:

Comment 1: First, the title needs to indicate efficacy and safety and the clinical research design of this study such as a prospective cohort study. The authors need to consider whether it is appropriate to describe it as a real-world study since real-world studies are often characterized by large samples. In fact, the authors analyzed cases from real-world clinical settings.
Reply 1: Thank you for your guidance. We indeed conducted an analysis based on cases from real-world clinical settings, encompassing a small sample size that aligns with the nature of observational studies in the real world. Our data primarily originated from treatment responses and survival events documented during routine clinical practice. In consideration of these aspects, we revised the title to '**Efficacy and safety of tucidinostat in patients with advanced hormone receptor-positive human epidermal growth factor receptor 2-negative breast cancer: real-world insights**.' Should you have any further suggestions or if additional clarification is needed, we're open to incorporating them to enhance the clarity of our response.
Changes in text: We revised the title " Efficacy and safety of tucidinostat in patients with advanced hormone receptor-positive human epidermal growth factor receptor 2-negative breast cancer: real-world insights." (page 1 line 3-4)

Comment 2: Second, the abstract needs some revisions. The authors did not describe the clinical needs for real-world data and the limitations of data from RCTs in the background. The methods need to describe the inclusion of subjects, the assessment of efficacy and safety, and follow up procedures. The conclusion needs some comments for the clinical implications of the findings.
Reply 2: Thank you for your suggestion. We have added a description of the RCT in the background. “However, existing evidence mainly stemmed from randomized controlled trials

(RCTs), and might have limitations in representing the complexities of clinical practice and diverse patient populations. Therefore, there is a need to explore the efficacy and optimal therapeutic modality for tucidinostat in real-world clinical settings." (page 2 line 41-44)
Changes in text: We have added relevant descriptions to the introduction. (page 2 line 41-44)

Comment 3: Third, the authors need to review and analyze the limitations and knowledge gaps of prior studies to support the need for real-world evidence. My major concern is the current data are not from a strictly deigned and retrospective, so the information from this study is not convincing.
Reply 3: Thank you for your inquiry. The enrolled patients were registered in the Breast Cancer Information Management System (BCIMS). Our data was not retrospective; it was collected regularly by clinical assistants through the BCIMS. Each month, these records were updated and verified within the hospital's electronic medical records. The efficacy and survival follow-up for each patient were relatively more accurate. Despite the small sample size, we believe this study still has certain clinical significance.

Comment 4: Fourth, please describe the calculation of sample size of this study and follow up details of this study. In statistics, please indicate the purposes of the Cox regression analysis.
Reply 4: Thank you for your inquiry. In our observational study, the calculation of sample size was not predetermined as our aim was to encompass all eligible cases during the study period, specifically including all patients receiving tucidinostat-based treatment for HR+/HER2- ABC. Clinical assistants conducted regular follow-ups on these patients through the Breast Cancer Information Management System (BCIMS), prospectively documenting various clinical and pathological characteristics, diagnoses, treatment courses, efficacy assessments, dates of death, or the last follow-up. We utilized Cox regression analysis to evaluate the impact of various factors on survival outcomes.
Changes in text:
We revised the description " We included all eligible patients who received tucidinostat therapy with histologically or cytologically confirmed HR+ HER2− ABC... " (page 5 line 126)
We revised the description "Clinical assistants conducted regular follow-ups on these patients through the Breast Cancer Information Management System (BCIMS), prospectively documenting various clinical and pathological characteristics, diagnoses, treatment courses, efficacy assessments, dates of death, or the last follow-up." (page 5 line 132-135)
We revised the description "The PFS and OS were estimated using the Kaplan-Meier method and compared with the log-rank test. Univariate Cox regression analysis was employed to assess the influencing factors for PFS." (page7 line 173-176)

Comment 5: Finally, please consider to review and cite several related papers: 1. Yuan Y, Zhang S, Wang T, Wang B, Wang S, Shi J, Sun T, Yin Y, Ouyang Q, Li J, Wen Y, Zhang L, Jiang Z. Efficacy and safety of abemaciclib-based therapy versus tucidinostat-based therapy after progression on palbociclib in patients with HR+HER2− metastatic breast cancer. Transl Breast Cancer Res 2023;4:10.. 2. Neven P, Dullens L, Han S, Deblander A, Van Herck Y, Van Houdt M, Wildiers H. Navigating next-generation HR+/HER2− metastatic breast cancer therapies: a critical commentary on abemaciclib vs. tucidinostat after palbociclib progression.

Transl Breast Cancer Res 2023;4:31. 3. Balanchivadze N, Robert NJ. Abemaciclib-based therapy versus tucidinostat-based therapy in patients with HR+HER2− metastatic breast cancer after palbociclib progression: insights and challenges from a comparative cohort study in China. Transl Breast Cancer Res 2023;4:32.. 4. Zhang Q, Li W, Hu X, Sun T, Cui S, Wang S, Ouyang Q, Yin Y, Geng C, Tong Z, Cheng Y, Ning Z, Jiang Z. Tucidinostat plus exemestane for postmenopausal patients with advanced, hormone receptor-positive breast cancer: a long-term safety and overall survival update from the randomised, double-blind, placebo-controlled, phase 3 trial. Transl Breast Cancer Res 2023;4:18.

Reply 5: Thank you for your suggestion. We cited this article in the introduction and discussion section.

Changes in text:

The updated results of ACE study showed the PFS extension did not translate into overall survival (OS) benefit(19). (page 4 line 103-105)

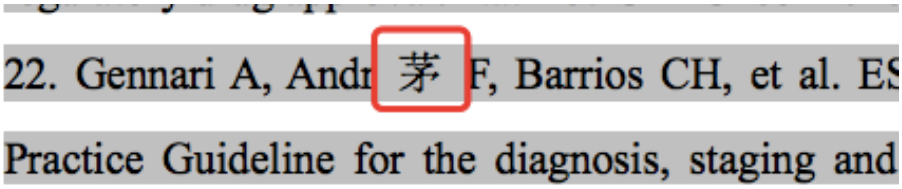
The conclusions drawn from a multicenter retrospective study recently did not strongly support tucidinostat. This study included HR+/HER2− ABC patients who experienced disease progression during palbociclib treatment. It aimed to compare the treatment efficacy of abemaciclib versus tucidinostat, revealing a significant extension in PFS within the abemaciclib group compared to the tucidinostat group (5.0 months vs. 2.0 months; $P < 0.001$) (30). The inherent heterogeneity in the patient population, such as the slightly higher proportion of patients non-sensitive to prior palbociclib and the lower use of fulvestrant in tucidinostat group, might partially explain the rapid disease progression observed in tucidinostat group (31,32). (page 9 line 300-303, page 10 line 304-308)

Reviewer D

1. Reference

a. There are two reference lists in the paper. Please deleted the unnecessary one.

b. Please revise.

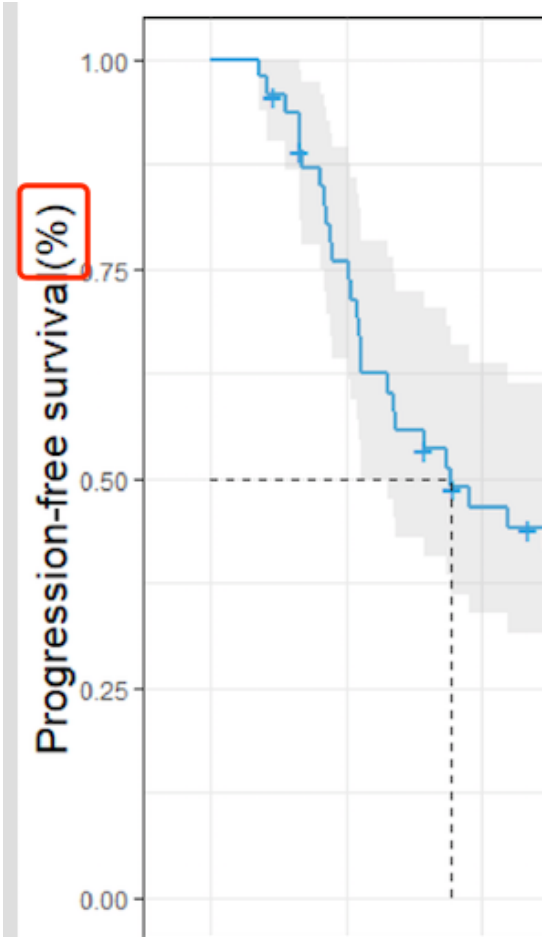

22. Gennari A, Andr 茅 F, Barrios CH, et al. ES

Practice Guideline for the diagnosis, staging and

Reply: We have removed the redundant reference list and corrected the errors in reference 22.

2. Figure 1 and Figure 2

Since the numbers in the Y-axis is [0-1], please remove the unit (%).



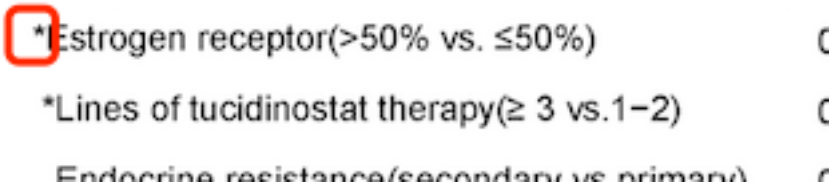
Reply: We have removed the percent sign from the diagram and renamed the picture to Figure 1 PFS-revised.

3. Figure 3

a. Please add (95% CI) after HR.

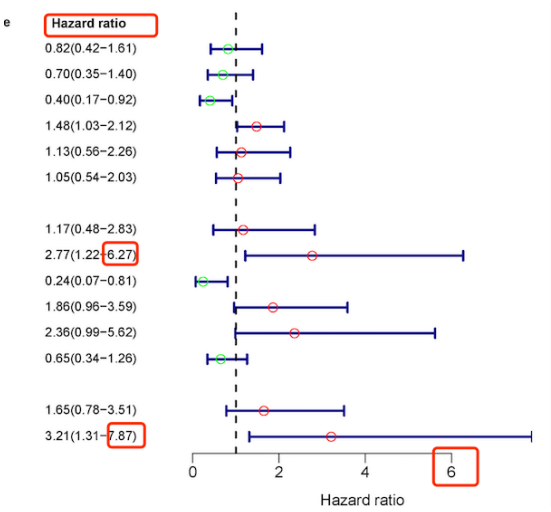
Reply: We added HR (95%CI) to Figure 3.

b. Please indicate the meaning of (*) in the figure legend.

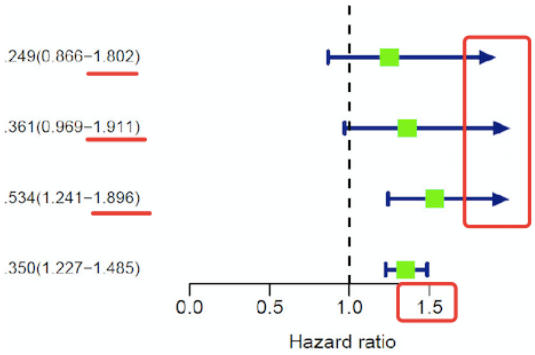


Reply: We have added the meaning of * in the figure legend.

c. To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows, or please extend the X-axis.



Here is an example:



Reply: We have extended the X-axis of figure3 and renamed it.

4. **When using abbreviations** in table/figure or table/figure description, please mention the entire expression in a footnote below the corresponding table/figure. Please check and revise. Such as: ER, PR, (in table 1); PD, (in table 2); PFS, (in table 3); etc.

Reply: We have added the entire expression of the abbreviation in the footnote