Peer Review File

Article information: https://dx.doi.org/10.21037/tbcr-23-9

Reviewer Comments

Reviewer A

Comment 1: A thorough linguistic revision through a native speaker should take place. just to name a few examples:
- Line 46-47: "no clinical data has been reported on which …" Please rephrase.
- Line 109-113: "As a highly selective CDK4/6i … reduction the risk of recurrence …" Please rephrase this sentence or rather build 2 sentences.
- Line 120-121: "… on which… "
- -Line 208: "More patients received sequential treatment in Abemaciclib group than that in tucidinostat group".
- Line 214 "of total patients " better: "of all patients"

Reply 1: Thank you for reviewer's suggestion, and I have made some modifications. I have revised the sentence in the 2nd paragraph line 9 of the introduction, 1st paragraph 6-7 line and 2nd paragraph 3 line of the results.

Comment 2: Line 81: Switching to a another CDK4/6i or tucidinostat is not recommended in other countries than China. We ask you to revise this statement and adapt it accordingly.

Reply 2: For post-CDK4/6i treatment, there is no standard of care according to international and domestic guidelines. Tucidinostat, an oral subtype-selective histone deacetylase inhibitor, showed active antitumor performance in patients who have progressed on prior endocrine therapy in ACE study, and was approved for patients with HR+HER2-MBC in China in 2019. With an increasing number of patients who progressed on CDK4/6i, tucidinostat-bsed therapy and a second course of CDK4/6i are commonly used in clinical practice in China, and are recommended as considerable treatment options post-CDK4/6i according to CSCO BC guidelines. Up to now, no clinical data has been reported on which of the two treatment strategies is more effective. Thus, we performed this study to evaluate the efficacy and safety of abemaciclib-based therapy and tucidinostat-based therapy after progression on palbociclib. Although tucidinostat is the only histone deacetylase inhibitor approved for patients with HR+HER2-MBC, other histone deacetylase inhibitors such as entinostat is under research and development. The result of our study may provide important data for efficacy and safety of histone deacetylase inhibitors after progression on CDK4/6i.

Comment 3: Line 147: What does the author mean by "discontinuation due to non-disease progression"? Discontinuation because of side effects? Or termination of therapy at the patient's request? Please explain more precisely.

Reply 3: Our study focused on patients who developed disease progression on palbociclb, and excluded patients who discontinued palbociclib due to non-disease progression. Discontinuation due to non-disease progression referred to discontinuation

because of side effects, and other non-medical factors such as patien's request.

Comment 4: Line 158: I think there is an error: “Abemaciclib-based therapy composed tucidinostat group”. “Tucidinostat-based therapy composed tucidinostat group”?
Reply 4: I have revised the sentence in 4th paragraph 2-3 line of the methods.

Comment 5: Line 164: Please define secondary endpoint “safety” more precisely.
Reply 5: We have defined safety in the last sentence in 5th paragraph of the methods. For each patient, the frequency and severity of adverse events and laboratory abnormalities (CTCAE version 4.03) occurred during the treatment course was recorded.

Comment 6: Line 184 ff: Variables which had been statistically significant in univariate Cox model and were considered clinically important were included for multivariate Cox regression. All univariate significant variables should be included in the multiple analysis, not only the clinically important ones as it can lead to different results. If this was not done, please repeat the analysis.
Reply 6: In univariate and multivariate analyses, we included almost all of the baseline characteristic factors. Variables which had been statistically significant in univariate Cox model were included for multivariate Cox regression, and ultimately these factors were included in the subgroup analysis. The univariate and multivariate analyses were shown in table 1.

Comment 7: Line 193-194: What does “sequential or non-sequential use” mean?
Reply 7: Sequential use in our study means that Abemaciclib/tucidinostat-based therapy was used immediately after palbociclib-based therapy. Non-sequential use means receiving one or several other treatments after palbociclib-based therapy and before Abemaciclib/tucidinostat-based therapy.

Comment 8: Line 206: What does “>3 metastatic sites” mean? – 3 different sites (e.g., bone, liver, lung) or 3 different sites only in the bone? Please be more specific.
Reply 8: We mean different metastatic organs. We have modified “sites” to “organs”.

Comment 9: Line 208: What does it mean: “More patients received sequential treatment in Abemaciclib group than that in tucidinostat group”. Please explain.
Reply 9: This sentence means A higher proportion of patients in abemaciclib group (36/73, 49.3%) received sequential treatment compared with that in tucidinostat group (23/76,30.3%). We have modified this sentence to make it easier to understand.

Comment 10: Line 219-221: What happened to the other 5 patients in Abemaciclib group and 2 patients in tucidinostat group? Abemaciclib: 73 patients total, 53 patients disease progression/dead, 15 patients still receiving treatment; 5 patients missing ??? Lost of follow-up? Please explain.
Reply 10: 5 patients in abemaciclib group and 2 patients in tucidinostat group were lost

of follow up.

Comment 11: It would be interesting to know which line of therapy the patients received abemaciclib or tucidinostat. Perhaps this information can be added to the results section?
Reply 11: Abemaciclib/tucidinostat-based therapy was median second line of endocrine therapy in MBC setting for the patients. We described the number of previous lines for MBC in the baseline features and added this information in table 1.

Comment 12: Furthermore, how many patients had primary metastatic disease and how many have secondary metastatic disease (metastatic disease after suffering locally breast cancer)? Is this already considered in the statistical analyses? If not- it might be a possible confounder as well.
Reply 12:

Distant relapse-free interval			**0.50**
De novo stage IV	**10 (13.7)**	**13 (17.1)**	
< 24 m	**11 (15.1)**	**7 (9.2)**	
≥24 m	**52 (71.2)**	**56 (73.7)**	

We described the number and proportion of patients with primary metastatic disease and secondary metastatic disease in Table 1. Thank you for your suggestion, we have included this factor in univariate Cox model and multivariate Cox regression, and subgroup analyses. However, the number of patients with de novo stage and DRFI < 24 months were small.

Comment 13: Please explain the rationale of the investigation of PFS according to PIK3CA mutation in the two groups. Was there a difference to be expected? Please explain this background using current literature.
Reply 13: Approximately 30–40% of advanced ER+ breast cancers have an activating PIK3CA mutation, previous studies have shown that these mutations lead to increased activation of the signaling pathway. All of these genetic alterations lead to hyperactivation of the PI3K/AKT/mTOR pathway thereby promoting cell transformation, tumor initiation, and resistance to apoptosis. Preclinical studies as well as retrospective analysis of some clinical trials in the metastatic setting have also suggested that ER+/PIK3CA mutant tumors have a lower response to anti-estrogens compared to ER+/PIK3CA wild-type tumors.
In the total population of our study, we found that abemaciclib-based therapy was superior to tucidinostat-based therapy in PFS, and PIK3CA gene mutations occurred in 44.2% of total patients. Since mutations in the PIK3CA gene mutations were associated with lower response to anti-estrogens, tumor initiation, and resistance to apoptosis, we assessed efficacy of abemaciclib-based therapy versus tucidinostat-based therapy in

PIK3CA-mutant and PIK3CA wild-type patients, and we found that in patients with PIK3CA-mutant, there was no significant difference in PFS between abemaciclib group and tucidinostat group (Figure 3B). In patients with PIK3CA wild-type, median PFS was 6.0 months in abemaciclib group and 2.0 months in tucidinostat group, but the difference was not significant (Figure 3C). PIK3CA-mutant showed a negative effect on PFS of abemaciclib-based therapy, which indicated that PIK3CA gene mutation may lead to lower response to the combination of abemaciclib and endocrine therapy, and that multigene sequencing in metastatic biopsy tissue while progression on CDK4/6i should be strongly considered.

Comment 14: Line 279-285: First part of the discussion section is a repetition of the results section and can be shortened/removed.
Reply 14: We removed some sentences in the first part of the discussion section according to your advice.

Comment 15: Line 292-293: ACE study. Reference is missing.
Reply 15: We added the reference of the ACE study.(16)

Comment 16: Line 312-315: Reference is missing.
Reply 16: Although palbociclib and abemaciclib have similar pharmacological effects, abemaciclib has its unique properties, including increased selectivity for CDK4 over CDK6, inhibiting CDK4/6 at low nanomolar concentrations, continuous administration, which have led to remarkable clinical performance in early-stage and metastatic breast cancer(21-23). References are number 21-23.

Comment 17: Line 312-318: The two sentences contradict each other. On the one hand, the anti-tumor effect of abemaciclib is pointed out by the author, which works independently of the endocrine pathway. On the other hand, reference is made to the simultaneous inhibition of the estrogen receptor. This should be explained in more detailed and provided with the appropriate references.
Reply 17: In this paragraph, we analyzed the reasons for the effectiveness of a second course of CDK4/6i-based therapy. On the one hand, the anti-tumor performance of the single-agent abemaciclib has been confirmed and approved by FDA, indicating that its single-agent anti-tumor performance may not depend on endocrine pathways. On the other hand, simultaneous inhibition of estrogen receptor and CDK4/6-cyclinD-Rb signaling pathway are important main drivers of cancer cell growth and survival in HR+HER2- tumors, targeting the two pathways may have a synergistic effect. In short, abemaciclib can act as a single agent without relying on endocrine pathways, and abemaciclib combined with endocrine therapy may perform more active anti-tumor therapeutic effects.

Comment 18: Why is line 324-332 marked blue?
Reply 18: Perhaps because it was a modified version.

Comment 19: Line 327-332: Please add references!!
Reply 19: Combined with the results of Cox analysis and subgroup analysis, patients receiving abemaciclib-based therapy and sequential use of abemaciclib/tucidinostat-based therapy after progression on palbociclib were associated with better clinical outcome in terms of PFS, and superiority of PFS in abemaciclib group was consistent across most subgroups, especially among patients with refractory factors.
These are the analysis of the results and no references. We added "in our study" in the beginning of this paragraph.

Comment 20: How many were pre-treated with chemotherapy? This should be mentioned in the text and considered as an influencing factor for the two groups.
Reply 20: The median lines of prior chemotherapy for MBC in both groups was 1. In fact, number of previous endocrine therapy lines is an important baseline characteristic, and we included this factor in univariate and multivariate analyses, and subgroup analyses.

Comment 21: Figure 2: Please explain what the numbers (e.g., 43? / 57?) mean and add this to the legend. How did you generate these numbers? -Are they absolute values / mean/ median? □ Please complete and explain.

		Events/patients		
		ET+Abemaciclib	ET+Tucidinostat	
Age,years				
	<60	43/57	54/57	⊢
	≥60	10/16	16/19	⊢—
Hormone receptor status				
	ER+/PR+	43/60	63/68	⊢

Reply 21: 43/57 means that the number of patients with age < 60 in abemaciclib group was 57, of which 43 had developed disease progression events.

Comment 22: Figure 2: Please explain abbreviations: 12m 12 months? Add in legend.
Reply 22: The information was missing, and we have added “m:months” in legend.

Comment 23: Figure 3: To make a final statement regarding the PFS in the case of PIK3CA mutation in the two groups, I consider it very risky given the small number of patients.
Reply 23: Your consideration is reasonable. We found that PIK3CA-mutant showed a negative effect on PFS of abemaciclib-based therapy. Although this is not our main research endpoint, the result may provide data for second course of CDK4/6i in patients with HR+HER2-MBC. After all, PIK3CA gene mutation occurs in a considerable

proportion of HR+HER2-MBC.
Comment 24: Table 1: Age was given as the median. Normally, range (min-max) should approximate the median. This should also be documented in the legend. What does the value in brackets mean (30.78) ?!

Reply 24: We added median(min ; max) in table 1, and added abbreviation in the legend.

Comment 25: Table 2: Please explain abbreviations: 6m □ 6 months? Furthermore, why is an additional percentage given for only half of the values? Please add accordingly.
Reply 25: We have modified table 2 according to your suggestion.

Comment 26: Table 3: Please explain abbreviations. "m"? "ref"?
Reply 26: We have modified table 3 according to your suggestion.

Reviewer B
Comment 1: This study is interesting because the optimal treatment for disease progression during the initial CDK4/6 inhibitor therapy remains undetermined.

Thus, explaining the tucidinostat administration in comparison to abemaciclib would be beneficial for this study. Please provide a simple history.
Reply 1: Thank you for your suggestion. There is a considerable high proportion of dose reduction in the daily use of tucidinostat due to adverse reactions. In our study, Table 2 shows that nearly one-fifth of patients use the minimum of 20mg tucidinostat as initial dose. In addition, the proportion of redused dosage and drug discontinuation of tucidinostat due to adverse reactions was also higher than abemaciclib. Insufficient doses of tucidinostat are likely to have a negative impact on efficacy.

Comment 2: In line 81, you stated "Switching another CDK4/6i or tucidinostat are reasonable treatment strategies for patients progressed on CDK4/6i." However, in the Discussion section, you stated "Our data indicated that addition of tucidinostat to ET did not show active anti-tumor performance on tumor progression during CDK4/6i. Tumor progressed during CDK4/6i and ET." This does not seem to fit with this study. Kindly review this and revise it appropriately.
Reply 2: Thank you for the reminder. For post-CDK4/6i treatment, there is no standard of care according to international and domestic guidelines. Tucidinostat combined with exemestane is superior to exemestane in patients who have progressed on prior endocrine therapy in ACE study. With an increasing number of patients with progression on CDK4/6i, tucidinostat-bsed therapy and a second course of CDK4/6i are commonly used in clinical practice, and are recommended as considerable treatment options post-CDK4/6i. Up to now, no clinical data has been reported on which of the two treatment strategies is more effective. Thus, we performed this study. We modified "reasonable treatment strategies" to "considerable treatment strategies".

Comment 3: Line 114: It would be better to state “was approved for patients with HR+HER2−MBC in China.”
Reply 3: We modified this sentence according to your suggestion.

Comment 4: Lines 157–158: In this sentence, “Patients who received abemaciclib-based therapy composed abemaciclib group, and those who received abemaciclib-based therapy composed tucidinostat group something,” I think it is tucidinostat instead of abemaciclib. Kindly review this carefully.
Reply 4: Thank you for the reminder. We modified this sentence according to your suggestion.

Comment 5: The repetition of the result is unnecessary.
Reply 5: We deleted the repetition of the result.