Peer Review File

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Reviewer comments	Author response and changes made	Page and line number where changes can be found in revised paper (highlighted in yellow)
REVIEWER A		
Seguì et al. wrote a remarkably exhaustive editorial commenting Tsuji's paper on the utility of whole-exome sequencing of liquid biopsies in patients receiving Bazedoxifene and Palbociclib. The paper competently summarizes most of the relevant findings, putting them in the context of the latest scientific evidences on next-generation SERDs and their clinical relevance for ESR1mut breast cancer. Seguì et. al also clearly highlight the many open questions and possible future research opportunities that such developments bring to our understanding of endocrine-resistance in breast cancer.	We are grateful for the positive comments.	-

I feel that the paper is well-written, clear, and concise, and suggest no corrections.		
REVIEWER B		
Cancer in the Era of Novel Endocrine Agents and CDK4/6-Inhibitors Great review	We thank the Reviewer for the positive comment.	
98- please clarify the combination you are referring to in this line or earlier in the paragraph, assuming you are referring to bazedoxifene+palbociclib	Regarding this comment, we were referring to the combination of bazedoxifene and palbociclib. The avoid possible confusion, we changed the paragraph as follows (changes in italics): These findings suggest that PIK3CA mutations could serve as potential biomarkers of resistance to *the combination of bazedoxifene and palbociclib*, and their relevance may extend to other next-generation endocrine therapies (page 3, lines 97-99)	Pg. 4, lines 106-107
101- consider separate paragraphs for PIK3CA and ESR for clarity	Regarding this comment, following the Reviewer's suggestion, we separated in a different paragraph the section regarding *ESR1*.	Pg. 4

REVIEWER C		
The editorial reviews a clinical study by Dr. Junko	We thank the Reviewer for its valuable feedback and constructive suggestions	-

Tsuji and colleagues which evaluated the efficacy and safety of bazedoxifene, a novel endocrine agent, in combination with palbociclib for the treatment of HR+/HER2- advanced breast cancer patients who have shown progression on prior endocrine treatment. The manuscript appears to be well-structured and detailed, shedding light on the importance of liquid biopsies to detect tumor heterogeneity, monitor genetic evolution, and identify actionable mutations during treatment. Please find below some minor points to be addressed:	on our manuscript	
1. While the safety aspect of the bazedoxifene and palbociclib combination is mentioned, a deeper exploration of the potential side effects and their consequences could enhance the paper's comprehensiveness	Regarding the first comment related to the safey aspect of bazedoxifene and palbociclib, we have expanded the safety section in our manuscript to provide a more detailed analysis of the safety profile associated with the new combination therapy. The new section is hereby reported: *In terms of safety, it's noteworthy that the addition of bazedoxifene to palbociclib did not exacerbate the adverse events previously known with palbociclib. The safety profile remained manageable throughout the study. Importantly, no dose-limiting toxicities were observed in the initial six patients enrolled in the safety run-in phase. Thus, 125mg dose palbociclib was used for the remainder of the study. In line with findings from the PALOMA-2 trial, the most commonly reported adverse events were any-grade neutropenia*	Pg. 3, lines 75-84

	(61%) and fatigue (22%)[12]. It is also worth highlighting that only one patient discontinued treatment due to protocol-specified unacceptable toxicity (neutropenia), and there were no treatment-related deaths reported in the study.[9] New reference 12 was added, as well: *Finn RS, et al. Palbociclib and Letrozole in Advanced Breast Cancer. New England Journal of Medicine. 2016; 375(20): 1925-1936)*	
2. The authors mention several ongoing studies. However, expanding on others, such as the ongoing INTERACT trial (NCT04256941), which investigates the efficacy between patients with ESR1 mutation treated with CDK4/6 inhibitors combined with either AI or fulvestrant, might further enrich the discussion;	Regarding the second comment, we appreciated the Reviewer's suggestions to include more information about the ongoing INTERACT trial. We have incorporated a brief mention to this trial in our manuscript. We also mentioned the ongoing SERENA-6. Hereby is reported the newly added section: *In this perspective, two ongoing randomized trials hold significance in this context. The phase II INTERACT (NCT04256941) aims to evaluate the PFS when transitioning to fulvestrant versus continuing AI therapy in patients treated with any CDK4/6 inhibitors with emergence of ESR1 mutations detected in plasma. Similarly, the phase III SERENA-6 trial (NCT04964934) explores whether switching to the oral SERD camizestrant while maintaining the same CDK4/6-inhibitor, upon detecting ESR1 mutations in ctDNA, improves PFS compared to continuing AI+CDK4/6-inhibitor until radiologic tumor progression.*	Pg. 5, lines 161-168

3. Please consider citing the guidelines provided by The ESMO Precision Medicine Working Group which refers to the usage of ctDNA in patients with cancers;	We appreciated Reviewer's suggestion. Thus, we have incorporated this citation into our manuscript to support and reinforce our discussion regarding the utility to ctDNA in advanced breast cancer management, as new ref. 22. *Pascual J, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Annals of Oncology. 2022;33(8):750-768*	
4. Although the editorial primarily underscores the role of ctDNA in metastatic settings, introducing a brief summary of the prospects of liquid biopsy for early cancer detection and its role in preliminary settings, would be enriching; 5. Please consider expanding the challenges and limitations of liquid biopsies in metastatic settings. In particular: a. Highlight that sensitivity may fluctuate depending on the methods employed; b. Point out that specific contexts, like oligometastatic or bone-only disease and the presence of brain metastases, might pose difficulties for ctDNA detection;	Regarding the fourth and fifth comment, we have included a brief section of the potential of liquid biopsy for early breast cancer and have expanded on the limitations of liquid biopsy. *While liquid biopsy offers valuable insights it also faces several limitations in its current application. It struggles to reliably detect fusion and copy number events, and it may produce false-negative results, even in advanced cancers, when time of acquisition is not carefully planned and when there is low ctDNA shedding, such as in cases of bone-only disease, oligometastatic disease or brain metastases. Additionally, false positives can occur due to clonal hematopoiesis[22].* *Whilst the role of liquid biopsy in ABC is well-established, emerging research is unveiling its potential to improve the management of early-stage breast cancer (EBC) by enabling non-invasive assessment of tumor burden. Serial ctDNA analysis in EBC can offer valuable insights for treatment decision-making, early assessment of treatment response and detecting minimal residual disease (MRD) or molecular relapse[28].*	Pg. 6, lines 189-200

c. Note that disease multifocality or bilaterality could introduce additional complications.	New reference 28 was added, as well: *Magbanua et al., Clinical significance and biology of circulating tumor DNA in high-risk early-stage HER2-negative breast cancer receiving neoadjuvant chemotherapy, Cancer Cell, 2023;41:1-23*	
6. The manuscript could provide readers with a clearer perspective by underscoring upcoming research trends in this area, including spotlighting the latest technological developments in ctDNA assays and discussing methods to more smoothly integrate liquid biopsy into standard clinical procedures.	Thanks to this insightful comment, we have added a paragraph highlighting the upcoming research trends and the latest technological advancements, as follows: *Future research in liquid biopsy should prioritize determining the ideal timing for dynamic ctDNA assessment and accurate threshold for response prediction both in the advanced and early settings. Additionally, efforts should be directed towards providing evidence of clinical utility for MRD assessment in the adjuvant setting and exploring the potential of liquid biopsy for screening of early-stage cancers and precancerous conditions in asymptomatic individuals. The development of novel technologies, such as methylation pattern-based sequencing, fragmentations pattern-based sequencing, and ultra-sensitive mutation detection, holds promise for optimizing liquid biopsy's utility in these emerging applications.* *Future research is warranted to explore how liquid biopsy can continue to offer clinically valuable insights for optimizing treatment strategies across all breast cancer patients.*	Pg. 7, lines 202-209 Pg. 7, lines 218-219