Liquid biopsy to tailor the treatment of advanced hormone receptor-positive breast cancer in the era of novel endocrine agents and CDK4/6-inhibitors

Elia Seguí[1,2,3]^, Francesco Schettini[2,3,4]^

[1]SOLTI Cancer Research Group, Barcelona, Spain; [2]Medical Oncology Department, Hospital Clinic of Barcelona, Barcelona, Spain; [3]Translational Genomics and Targeted Therapies in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (FRCB-IDIBAPS), Barcelona, Spain; [4]Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

Correspondence to: Francesco Schettini, MD, PhD. Faculty of Medicine and Health Sciences, University of Barcelona, C. Casanova 143, 08036 Barcelona, Spain; Medical Oncology Department, Hospital Clinic of Barcelona, Barcelona, Spain; Translational Genomics and Targeted Therapies in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (FRCB-IDIBAPS), Barcelona, Spain. Email: schettini@recerca.clinic.cat.

Comment on: Tsuji J, Li T, Grinshpun A, *et al.* Clinical Efficacy and Whole-Exome Sequencing of Liquid Biopsies in a Phase IB/II Study of Bazedoxifene and Palbociclib in Advanced Hormone Receptor-Positive Breast Cancer. Clin Cancer Res 2022;28:5066-78.

Keywords: CDK4/6-inhibitors; liquid biopsy; endocrine resistance; ESR1; bazedoxifene

Submitted Aug 08, 2023. Accepted for publication Oct 10, 2023. Published online Oct 30, 2023.
doi: 10.21037/atm-23-1804
View this article at: https://dx.doi.org/10.21037/atm-23-1804

Multiple clinical trials and real-world evidence studies have consistently shown the remarkable efficacy of CDK4/6-inhibitors in combination with endocrine therapy (ET) as first/second-line treatment of hormone receptor-positive (HR^+)/HER2-negative ($HER2^-$) advanced breast cancer (ABC) (1-3). These drugs in combination with either an aromatase inhibitor (AI) or the selective estrogen receptor degrader (SERD) fulvestrant, significantly prolonged disease control, overall survival, and improved quality of life compared to ET alone or most chemotherapy regimens, proving to be effective also in case of visceral crisis (3-6). However, despite the significant advancements achieved, HR^+/$HER2^-$ ABC is still an incurable disease, with progression on CDK4/6-inhibitor + ET ultimately occurring, sooner or later.

Recent findings have highlighted the importance of refining the targeting of the estrogen receptor (ER) to improve the synergy between ET and CDK4/6-inhibitors, in order to achieve better outcomes. In this perspective, novel endocrine agents such as oral SERDs, selective ER modulators (SERMs) and proteolysis targeting chimeras (PROTACs) specific to ER are under active development as potential novel endocrine agent partners for CDK4/6-inhibitors, as well as in monotherapy or in combination with other target agents (7,8).

In their recent article on *Clinical Cancer Research*, Dr. Tsuji and colleagues presented the results of a phase IB/II single-arm trial evaluating the role of the novel endocrine agent bazedoxifene in combination with palbociclib in patients with HR^+/$HER2^-$ ABC who had progressed on prior endocrine treatment (NCT02448771) (9). Bazedoxifene is a third-generation SERM and SERD hybrid that has demonstrated activity in preclinical models of endocrine-resistant breast cancer, including models harboring *ESR1* mutations (10), and has shown a favourable safety profile in clinical trials with healthy women (11). In fact, it is currently approved for the treatment of postmenopausal osteoporosis in women at increased risk of fracture. The study from Tsuji *et al.* enrolled 36 patients with HR^+/$HER2^-$ ABC who had experienced disease progression on prior ET. There was no restriction on the number of prior ET, and up to 1–2 previous lines of

^ ORCID: Elia Seguí, 0000-0001-7770-9825; Francesco Schettini, 0000-0001-6561-1919.

chemotherapy were allowed. Notably, most patients had liver metastases (64%), with 50% having received at least two lines of prior ET and 52% having undergone previous chemotherapy in the advanced setting. All patients received bazedoxifene 40 mg orally once daily on days 1–28 and palbociclib in standard dosing.

The trial successfully achieved its primary endpoint with a clinical benefit rate of 33.3% [1 partial response (PR) and 11 stable diseases (SD) ≥24 weeks], surpassing the predetermined threshold of at least 11 patients experiencing clinical benefit. Of note, four patients had an outstanding clinical benefit with disease stability >12 months. The number of prior treatment regimens in the advanced setting did not correlate with clinical benefit. 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, 125 mg 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 (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).

While the trial's main objective was to provide activity and safety data for the bazedoxifene and palbociclib combination to allow for its further development, the study provided an interesting set of translational biomarker analyses, as well (9). A serial plasma collection performed at baseline (n=36), day 1 of cycle 2 (C2D1, n=33) and at the time of disease progression [end of treatment (EOT), n=33] was carried out to evaluate cell-free DNA (cfDNA) tumor fraction (TF) and presence of tumor somatic mutations. When cfDNA TF in a sample was superior to 0.03, the sample was considered to be positive for circulating tumor DNA (ctDNA). Although no significant association with progression-free survival (PFS) was observed between the early changes in cfDNA TF after 1 month of treatment, baseline cfDNA TF showed significant association with tumor burden (the higher the TF, the higher the number of affected organs) and PFS (the lower the TF, the better the outcome) (9). To note, despite different methodological approach, our group and others demonstrated that ctDNA might be useful to predict therapeutic outcomes in ABC treated with CDK4/6-inhibitors and ET, as well (13,14).

In 68 (66.7%) samples with detectable ctDNA, whole-exome sequencing (WES) was performed to identify mutations potentially associated with treatment sensitivity and resistance. The most frequently observed mutations at baseline involved *ESR1* and *PIK3CA*. Baseline activating *PIK3CA* mutations were not found in patients who had a clinical benefit and were associated with shorter PFS, in line with other studies (15), while baseline activating *ESR1* mutations were found in patients with and without clinical benefit from the combination and had no impact on PFS. 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. To note, *PIK3CA*-mutant HR$^+$/HER2$^-$ ABC can be already targeted with the alfa-selective PI3K inhibitor alpelisib + ET, and more PI3K inhibitors are under development, as well (16). On the other hand, the presence of *ESR1* mutations did not impair the efficacy of bazedoxifene and palbociclib.

ESR1 is the gene codifying for ER, and activating mutations occurring in the ligand binding domain can be found in 20–40% of patients with metastatic HR$^+$ tumors who have received an AI (17). These mutations stabilize ER's active conformation, resulting in the development of endocrine resistance. In a combined biomarker analysis of the SoFEA and EFFECT phase III trials of fulvestrant +/– AI *vs.* AI, baseline *ESR1* mutations detected in ctDNA impaired AI efficacy but not that of fulvestrant (17). In the more recent PADA-1 trial, patients with HR$^+$/HER2$^-$ ABC harboring an *ESR1*-activating mutation had twice the odds of disease progression when treated with an AI + palbociclib compared to those with wild-type *ESR1*. Interestingly, patients in this study with rising *ESR1* mutations in ctDNA experienced improved PFS when switched to fulvestrant and palbociclib, as opposed to continuing AI as ET backbone (18). New oral SERDs like elacestrant or camizestrant also proved to be particularly effective, and superior to fulvestrant, in *ESR1*-mutant HR$^+$/HER2$^-$ ABC, as detected in ctDNA (7,19). These and other novel agents are currently under investigation in combination with CDK4/6-inhibitors (7).

Considering bazedoxifene's superior activity in inhibiting the most common *ESR1* mutations compared to fulvestrant in preclinical models (10,20), along with the efficacy outlined by Tsuji *et al.* in their phase I/II study (9); provided also the superior efficacy of novel endocrine agents in *ESR1*-mutant tumors compared to fulvestrant, bazedoxifene

might warrant further investigation as CDK4/6-inhibitor partner in *ESR1*-mutant HR$^+$/HER2$^-$ ABC. In this scenario, liquid biopsy appeared to be potentially useful to both identify a subset of patients with *PIK3CA* mutations to whom this therapeutic approach should not be proposed and *ESR1*-mutant cases suitable for bazedoxifene.

Noteworthy, the comparison between EOT ctDNA and baseline ctDNA revealed that several patients acquired actionable driver mutations (i.e., *BRAF* missense mutations, *PIK3CA* hotspot mutations, *ERBB2* mutations) throughout the study treatment (9). These mutations provide valuable insights into potential mechanisms of acquired resistance and could serve as therapeutic targets, either in clinical practice or within clinical trials. Moreover, it was observed that the presence of the APOBEC signature was associated with a lack of clinical benefit from the combination of bazedoxifene and palbociclib, supporting previous evidence suggesting a correlation between this genomic signature and endocrine resistance (21).

Taken together, all these data add further evidence to support the implementation of liquid biopsy in the clinical practice of HR$^+$/HER2$^-$ ABC, especially when it comes to the prescription of novel ET agents or targeted therapies (22). Liquid biopsy compared to traditional tissue biopsies, offers the advantage of being minimally invasive, provides a more comprehensive assessment of tumor heterogeneity by capturing genetic alterations from multiple tumor sites simultaneously and allows for real-time monitoring of tumor biology evolution and treatment response. In fact, the detection of specific mutations in ctDNA for the prescription of targeted therapeutic approaches is already a reality in HR$^+$/HER2$^-$ ABC, with the prescription of alpelisib or elacestrant subject to the identification of *PIK3CA* or *ESR1* mutations, respectively (22). In their study, Tsuji *et al.* provide further evidence that tracking tumor sub-clonal evolution under therapeutic selective pressure is feasible and promising, since several actionable mutations were identified.

Apart from this, there is a huge interest in testing the possibility of liquid biopsy as tool to detect the emergence of therapeutic resistance and promote therapeutic decision-making before radiologic progression. This might enable a timelier adjustment to therapy, as hinted by the PADA-1 trial results (18). 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. If positive, these results might open to the possibility of anticipating therapeutic changes according to a seriated monitoring of tumor mutational profile through liquid biopsy, radically changing the way we currently treat metastatic breast cancer.

Nevertheless, while accumulating evidence supports liquid biopsies as a valuable tool for identifying individual genomic alterations, it is important to acknowledge that breast cancer is a complex disease, which behaviour is determined in most cases by multiple genomic features, rather than single driver genomic mutations (23). While single DNA alterations in tumor cells remain clinically useful, it is essential to consider that refining patients' prognosis and treatment outcomes may require additional biological information. Phenotypic characterization through multi-gene RNA-based expression analysis could offer valuable insights, for example by detecting the prognostic and predictive molecular intrinsic subtypes (23-26). Nonetheless, implementing tumor-based RNA-based gene expression profiling in the metastatic setting poses significant challenges due to limited availability of tumor tissue, tumor heterogeneity, and evolving tumor biology during treatment. In this context, a recent study by Prat *et al.* convincingly demonstrated the capability of plasma ctDNA to capture intricate tumor phenotypes, track breast cancer biological processes and identify distinct tumor subtypes within HR$^+$/HER2$^-$ ABC, by using a methodologically novel multi-gene signature approach, which might also predict the response to CDK4/6-inhibitor-based regimens (27).

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).

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.

In summary, the study from Tsuji *et al.* showed that the combination of bazedoxifene and palbociclib is safe and potentially active in endocrine-pretreated HR+/HER2− ABC, with or without *ESR1* mutations, further supporting its clinical development. This trial also strengthened the evidence to support the use of liquid biopsies to capture tumor heterogeneity, track subclonal genetic evolution, and identify actionable mutations acquired during treatment, shedding a light on the potential of liquid biopsies as a valuable tool for guiding treatment decisions in the evolving therapeutic landscape of HR+/HER2− ABC. 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.

Acknowledgments

The study was supported by a Rio Hortega contract from the Instituto de Salud Carlos III (ISCIII) to F.S. However, the findings and hypothesis generated are solely of the article's authors.
Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Annals of Translational Medicine*. The article has undergone external peer review.

Peer Review File: Available at https://atm.amegroups.com/article/view/10.21037/atm-23-1804/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-23-1804/coif). E.S. reports having obtained travel/accommodation paid by Gilead, Daiichi Sankyo, Novartis and Lilly, and personal fees for educational events from Novartis and Pfizer. F.S. reports having obtained travel/accommodation paid by Novartis and Gilead, and personal fees for educational events from Novartis, Daiichy-Sankyo and Gilead. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Harbeck N, Bartlett M, Spurden D, et al. CDK4/6 inhibitors in HR+/HER2- advanced/metastatic breast cancer: a systematic literature review of real-world evidence studies. Future Oncol 2021;17:2107-22.
2. Gao JJ, Cheng J, Bloomquist E, et al. CDK4/6 inhibitor treatment for patients with hormone receptor-positive, HER2-negative, advanced or metastatic breast cancer: a US Food and Drug Administration pooled analysis. Lancet Oncol 2020;21:250-60.
3. Schettini F, Giudici F, Giuliano M, et al. Overall Survival of CDK4/6-Inhibitor-Based Treatments in Clinically Relevant Subgroups of Metastatic Breast Cancer: Systematic Review and Meta-Analysis. J Natl Cancer Inst 2020;112:1089-97.
4. Giuliano M, Schettini F, Rognoni C, et al. Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network

meta-analysis. Lancet Oncol 2019;20:1360-9.
5. Schettini F, Seguí E, Conte B, et al. Intrinsic subtypes and therapeutic decision-making in hormone receptor-positive/HER2-negative metastatic breast cancer with visceral crisis: A case report. Front Oncol 2022;12:1009352.
6. Lu YS, Bin Mohd Mahidin EI, Azim H, et al. Primary results from the randomized Phase II RIGHT Choice trial of premenopausal patients with aggressive HR+/HER2– advanced breast cancer treated with ribociclib + endocrine therapy vs physician's choice combination chemotherapy [abstract]. In: Proceedings of the 2022 San Antonio Breast Cancer Symposium; 2022 Dec 6-10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2023;83:Abstract nr GS1-10.
7. Garcia-Fructuoso I, Gomez-Bravo R, Schettini F. Integrating new oral selective oestrogen receptor degraders in the breast cancer treatment. Curr Opin Oncol 2022;34:635-42.
8. Hernando C, Ortega-Morillo B, Tapia M, et al. Oral Selective Estrogen Receptor Degraders (SERDs) as a Novel Breast Cancer Therapy: Present and Future from a Clinical Perspective. Int J Mol Sci 2021;22:7812.
9. Tsuji J, Li T, Grinshpun A, et al. Clinical Efficacy and Whole-Exome Sequencing of Liquid Biopsies in a Phase IB/II Study of Bazedoxifene and Palbociclib in Advanced Hormone Receptor-Positive Breast Cancer. Clin Cancer Res 2022;28:5066-78.
10. Fanning SW, Jeselsohn R, Dharmarajan V, et al. The SERM/SERD bazedoxifene disrupts ESR1 helix 12 to overcome acquired hormone resistance in breast cancer cells. Elife 2018;7:e37161.
11. Duggan ST, McKeage K. Bazedoxifene: a review of its use in the treatment of postmenopausal osteoporosis. Drugs 2011;71:2193-212.
12. Finn RS, Martin M, Rugo HS, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med 2016;375:1925-36.
13. Martínez-Sáez O, Pascual T, Brasó-Maristany F, et al. Circulating tumor DNA dynamics in advanced breast cancer treated with CDK4/6 inhibition and endocrine therapy. NPJ Breast Cancer 2021;7:8.
14. Malorni L, Bianchini G, Caputo R, et al. Serum thymidine kinase activity in patients with HR-positive/HER2-negative advanced breast cancer treated with ribociclib plus letrozole: Results from the prospective BioItaLEE trial. Eur J Cancer 2023;186:1-11.
15. Mosele F, Stefanovska B, Lusque A, et al. Outcome and molecular landscape of patients with PIK3CA-mutated metastatic breast cancer. Ann Oncol 2020;31:377-86.
16. Sirico M, D'Angelo A, Gianni C, et al. Current State and Future Challenges for PI3K Inhibitors in Cancer Therapy. Cancers (Basel) 2023;15:703.
17. Turner NC, Swift C, Kilburn L, et al. ESR1 Mutations and Overall Survival on Fulvestrant versus Exemestane in Advanced Hormone Receptor-Positive Breast Cancer: A Combined Analysis of the Phase III SoFEA and EFECT Trials. Clin Cancer Res 2020;26:5172-7.
18. Bidard FC, Hardy-Bessard AC, Dalenc F, et al. Switch to fulvestrant and palbociclib versus no switch in advanced breast cancer with rising ESR1 mutation during aromatase inhibitor and palbociclib therapy (PADA-1): a randomised, open-label, multicentre, phase 3 trial. Lancet Oncol 2022;23:1367-77.
19. Oliveira M, Pominchuk D, Hamilton EP, et al. Clinical activity of camizestrant, a next-generation SERD, versus fulvestrant in patients with a detectable ESR1 mutation: Exploratory analysis of the SERENA-2 phase 2 trial. J Clin Oncol 2023;41:1066.
20. Wardell SE, Nelson ER, Chao CA, et al. Evaluation of the pharmacological activities of RAD1901, a selective estrogen receptor degrader. Endocr Relat Cancer 2015;22:713-24.
21. Marra A, Gazzo A, Gupta A, et al. 210O Mutational signature analysis reveals patterns of genomic instability linked to resistance to endocrine therapy (ET) +/- CDK 4/6 inhibition (CDK4/6i) in estrogen receptor-positive/HER2-negative (ER+/HER2-) metastatic breast cancer (MBC). Ann Oncol 2022;33:S632.
22. Pascual J, Attard G, Bidard FC, 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. Ann Oncol 2022;33:750-68.
23. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
24. Schettini F, Brasó-Maristany F, Kuderer NM, et al. A perspective on the development and lack of interchangeability of the breast cancer intrinsic subtypes. NPJ Breast Cancer 2022;8:85.
25. Falato C, Schettini F, Pascual T, et al. Clinical implications of the intrinsic molecular subtypes in hormone receptor-positive and HER2-negative metastatic breast cancer. Cancer Treat Rev 2023;112:102496.
26. Schettini F, Martínez-Sáez O, Falato C, et al. Prognostic value of intrinsic subtypes in hormone-receptor-positive metastatic breast cancer: systematic review and meta-

analysis. ESMO Open 2023;8:101214.
27. Prat A, Brasó-Maristany F, Martínez-Sáez O, et al. Circulating tumor DNA reveals complex biological features with clinical relevance in metastatic breast cancer. Nat Commun 2023;14:1157.
28. Magbanua MJM, Brown Swigart L, Ahmed Z, 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;41:1091-1102.e4.

Cite this article as: Seguí E, Schettini F. Liquid biopsy to tailor the treatment of advanced hormone receptor-positive breast cancer in the era of novel endocrine agents and CDK4/6-inhibitors. Ann Transl Med 2024;12(1):19. doi: 10.21037/atm-23-1804