Plasma ESR1 Mutations and the Treatment of Estrogen Receptor–Positive Advanced Breast Cancer

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Purpose: ESR1 mutations are selected by prior aromatase inhibitor (AI) therapy in advanced breast cancer. We assessed the impact of ESR1 mutations on sensitivity to standard therapies in two phase III randomized trials that represent the development of the current standard therapy for estrogen receptor–positive advanced breast cancer.

Materials and methods: In a prospective-retrospective analysis, we assessed ESR1 mutations in available archived baseline plasma from the SoFEA (Study of Faslodex Versus Exemestane With or Without Arimidex) trial, which compared exemestane with fulvestrant-containing regimens in patients with prior sensitivity to nonsteroidal AI and in baseline plasma from the PALOMA3 (Palbociclib Combined With Fulvestrant in Hormone Receptor–Positive HER2-Negative Metastatic Breast Cancer After Endocrine Failure) trial, which compared fulvestrant plus placebo with fulvestrant plus palbociclib in patients with progression after receiving prior endocrine therapy. ESR1 mutations were analyzed by multiplex digital polymerase chain reaction.

Results: In SoFEA, ESR1 mutations were found in 39.1% of patients (63 of 161), of whom 49.1% (27 of 55) were polyclonal, with rates of mutation detection unaffected by delays in processing of archival plasma. Patients with ESR1 mutations had improved progression-free survival (PFS) after taking fulvestrant (n = 45) compared with exemestane (n = 18; hazard ratio [HR], 0.52; 95% CI, 0.30 to 0.92; \( P = .02 \)), whereas patients with wild-type ESR1 had similar PFS after receiving either treatment (HR, 1.07; 95% CI, 0.68 to 1.67; \( P = .77 \)). In PALOMA3, ESR1 mutations were found in the plasma of 25.3% of patients (91 of 360), of whom 28.6% (26 of 91) were polyclonal, with mutations associated with acquired resistance to prior AI. Fulvestrant plus palbociclib improved PFS compared with fulvestrant plus placebo in both ESR1 mutant (HR, 0.43; 95% CI, 0.25 to 0.74; \( P = .002 \)) and ESR1 wild-type patients (HR, 0.49; 95% CI, 0.35 to 0.70; \( P < .001 \)).

Conclusion: ESR1 mutation analysis in plasma after progression after prior AI therapy may help direct choice of further endocrine-based therapy. Additional confirmatory studies are required.

Introduction

Targeting the estrogen receptor (ER) with endocrine therapies was the first molecularly targeted treatment of breast cancer and remains a mainstay of treatment of all stages of ER-positive disease.1–3 Approximately 75% of breast cancers are ER-positive, with endocrine therapy the favored initial choice for patients who develop metastatic disease.4 In this setting, almost all patients will acquire endocrine resistance, with a proportion demonstrating primary resistance. Identifying therapies with activity in tumors resistant to standard endocrine therapy is a key therapeutic challenge.

Although diverse mechanisms of resistance to endocrine therapy have been described, recent evidence has identified mutations in the ER gene (ESR1).5 ESR1 mutations occur rarely in primary breast cancer,6 but have a high prevalence in advanced breast cancer.7

Key points

- Approximately 75% of breast cancers are ER-positive, with endocrine therapy the favored initial choice for patients who develop metastatic disease.
cancers previously treated with aromatase inhibitors (AIs), implying evolution through selective treatment pressure. Most ESR1 mutations occur in hotspot regions in the ligand-binding domain of ER, resulting in ligand-independent, constitutive ER activity. Prior research has demonstrated that circulating tumor DNA (ctDNA) is detected in the plasma of patients with cancer and may provide a robust, noninvasive method for detecting ESR1 mutations.

The most effective treatment of ESR1 mutant breast cancer is uncertain. In a retrospective, single-center analysis, we have demonstrated resistance to subsequent AI-based therapy in patients with ESR1 mutations in plasma. Preclinical studies have reported growth inhibition of ESR1 mutant cell lines with fulvestrant, a selective ER degrader, but with less sensitivity to fulvestrant than wild-type ER, and there is uncertainty whether the required doses are achieved clinically. Palbociclib, a CDK4/6 inhibitor, has demonstrated substantial clinical activity in combination with both fulvestrant and AIs. Preclinical studies have reported growth inhibition of ESR1 mutant cell lines with fulvestrant, a selective ER degrader, but with less sensitivity to fulvestrant than wild-type ER, and there is uncertainty whether the required doses are achieved clinically.

CDK4/6 inhibition resensitizes cells with in vitro-derived resistance to endocrine therapy, and ESR1 mutant models are sensitive to combinations of selective ER degraders with palbociclib.

From our prior retrospective study, we hypothesized that ESR1 mutant patients would have a poor prognosis when treated with exemestane and that prognosis would be improved with fulvestrant.

Materials and methods

The SoFEA study was a multicenter, randomized phase III trial in postmenopausal women with advanced, hormone receptor–positive breast cancer who had demonstrated prior sensitivity to AIs. Sensitivity was defined as relapse or progression after taking a nonsteroidal AI given as adjuvant treatment for at least 12 months or as first-line metastatic treatment for at least 6 months. Patients were assigned fulvestrant (500 mg intramuscularly on day 1, followed by 250 mg on days 15 and 29, then every 28 days) plus anastrozole 1 mg, fulvestrant plus placebo, or exemestane 25 mg. Baseline plasma samples were available from 162 patients of the 723 enrolled (22.4%), with no samples available before January 2, 2008, because of a fire at the Royal Marsden Hospital (Figure 1A). The subset of patients with baseline plasma samples available had similar characteristics, except for a longer time to relapse and a longer time taking an AI, and outcomes similar to the rest of the study population. Written informed consent was obtained from all participants, and ESR1 analysis was approved by the research ethics committee.

The PALOMA3 trial was a multicenter, randomized phase III trial assessing palbociclib and fulvestrant in premenopausal and postmenopausal women with advanced,
hormone receptor–positive breast cancer who had progressed during prior endocrine therapy. Patients were assigned 2:1 to palbociclib (125 mg orally for 3 weeks followed by 1 week off) and fulvestrant (500 mg intramuscularly every 14 days for the first three injections, then 500 mg every 28 days), or matching placebo plus fulvestrant. Premenopausal patients received goserelin for the study duration. We analyzed 360 baseline plasma samples from 521 patients (69.1%) enrolled in
• For ESR1 mutation analysis, we used commercially available multiplex droplet digital polymerase chain reaction (ddPCR) assays for the seven most common ESR1 mutations.

• ddPCR was performed on a QX200 system (Bio-Rad, Hercules, CA) on a minimum of 1-mL plasma equivalent for SoFEA samples and 0.5-mL for PALOMA3.

• A multiplex assay was considered mutation positive if at least two ESR1 mutant droplets were observed. The results obtained on the multiplex ddPCR were further characterized using uniplex ddPCR assays.

• A sample was considered polyclonal if it was positive on both multiplexes or if separate mutations were characterized on uniplex confirmation.

• In this prospective-retrospective study, the most recent clinical data snapshots were used for both SoFEA and PALOMA3.

• ESR1 mutation status was measured as a binary outcome (mutated vs. wild type).

• The principal analysis population for both trials included all patients who were randomly assigned on an intention-to-treat basis and had been assessed for ESR1 status.

• Survival end point comparisons were made using the log-rank test. Hazard ratios (HRs) were obtained from Cox proportional hazards regression models.

The PALOMA3 trial (Figure 1B). The subset of patients with baseline plasma samples available had similar characteristics, with the exception of prior chemotherapy exposure, and outcomes similar to the rest of the study population. Written informed consent was obtained from all participants.

- Processing of plasma and extraction of circulating DNA

In the SoFEA trial, baseline blood was collected in EDTA blood collection tubes and processed within 0 to 9 days of sample collection. Plasma was separated by centrifugation at 1,600 g for 20 minutes. In the PALOMA3 study, baseline blood was collected in EDTA tubes and centrifuged within 30 minutes at 1,500 to 2,000 g for 10 minutes. Samples were stored at –80°C until DNA extraction. DNA extraction was performed using the QiAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

- Digital polymerase chain reaction analysis

Total free DNA was quantified from plasma using RNase P as the reference gene as previously reported. For ESR1 mutation analysis, we used commercially available multiplex droplet digital polymerase chain reaction (ddPCR) assays for the seven most common ESR1 mutations; multiplex 1 included c.1138G>C(E380Q), c.1607T>G(L536R), c.1610A>G(Y537C), and c.1613A>G(D538G; dHsaM-DXE91450042); and multiplex 2 included c.1387T>C(S463P), c.1609T>A(Y537N), and c.1610A>C(Y537S; dHsaM-DXE65719815). ddPCR was performed on a QX200 system (Bio-Rad, Hercules, CA) on a minimum of 1-mL plasma equivalent for SoFEA samples and 0.5-mL for PALOMA3. A multiplex assay was considered mutation positive if at least two ESR1 mutant droplets were observed. The results obtained on the multiplex ddPCR were further characterized using uniplex ddPCR assays.

Mutation allele fraction and copies per mL were calculated as previously described. A sample was considered polyclonal if it was positive on both multiplexes or if separate mutations were characterized on uniplex confirmation.

- Validation of analysis of ESR1 mutations in archival plasma samples

Archival plasma samples were available in the SoFEA trial, and we validated the analysis of ctDNA in archival plasma, demonstrating that archival EDTA plasma samples can be used for ctDNA analysis with ddPCR.

- Statistical analysis

In this prospective-retrospective study, the most recent clinical data snapshots were used for both SoFEA and PALOMA3. ESR1 mutation status was measured as a binary outcome (mutated vs. wild type). The principal analysis population for both trials included all patients who were randomly assigned on an intention-to-treat basis and had been assessed for ESR1 status. There was no difference in efficacy between the two fulvestrant groups in the SoFEA trial, and these were merged for primary end point analysis, as prespecified in the statistical analysis plan. P values were two tailed and considered significant if P was <.05 for the principal analyses of PFS. Other analyses were exploratory and considered significant if P was <.01 to take multiple comparisons into account.

Survival end point comparisons were made using the log-rank test. Hazard ratios (HRs) were obtained from Cox proportional hazards regression models. The proportionality assumption of the Cox models was tested with Schoenfeld residuals and was shown to hold for all analyses. Survival data in PALOMA3 were stratified according to prior chemotherapy status, the presence or absence of visceral disease and sensitivity to prior endocrine therapy in line with the main trial analyses. Interaction tests were used to explore differential effects between ESR1 mutation status and...
trial treatment in relation to PFS. Multivariable models assessed ESR1 mutation status and treatment group separately for each trial, adjusting for, in the case of SoFEA, factors identified for the principal analysis, namely, time from diagnosis to first relapse, number of disease sites present at baseline, and prior AI setting and time receiving an AI. Any baseline characteristics that were statistically significant when comparing ESR1 mutation versus wild type were also included in the multivariable models separately for SoFEA and PALOMA3 if they significantly added value to the model (likelihood ratio test $P < .05$). All statistical analyses were performed with Stata (version 13.1; STATA, College Station, TX) or GraphPad Prism (version 6.0; GraphPad Software, La Jolla, CA).

### Results

Impact of ESR1 mutation on sensitivity to endocrine therapies in SoFEA

In SoFEA, ESR1 mutation status was successfully analyzed in 99.4% (161 of 162, representing 22.4% of all patients) of available baseline plasma samples, with ESR1 mutations detected in 39.1% of samples (63 of 161). We assessed the impact of ESR1 mutations on outcome in patients randomly assigned to receive exemestane ($n = 57$) versus fulvestrant-containing ($n = 104$) regimens. For patients with ESR1 mutant ctDNA, the median PFS was 2.6 months (95% CI, 2.4 to 6.2) for patients given exemestane and 5.7 months (95% CI, 3.0 to 8.5) for those given fulvestrant (Figure 2A; HR, 0.52; 95% CI, 0.30 to 0.92; $P = .02$), whereas patients with wild-type ESR1 had a median PFS of 8.0 months (95% CI, 3.0 to 11.5) when given exemestane and a median PFS of 5.4 months (95% CI, 3.7 to 8.1) when given fulvestrant (Figure 2B; HR, 1.07; 95% CI, 0.68 to 1.67; $P = .77$). The interaction test between treatment allocation and ESR1 mutation status was $P = .07$.

Considering ESR1 mutation status within the exemestane group, patients with an ESR1 mutation had worse PFS than ESR1 wild type (HR, 2.12; 95% CI, 1.18 to 3.81; $P = .01$). In the SoFEA study, the number of deaths provided insufficient statistical power to detect a statistically significant difference in survival curves, although the effects of ESR1 mutation on overall survival were considered.

**Key points**

- In SoFEA, ESR1 mutation status was successfully analyzed in 99.4% (161 of 162, representing 22.4% of all patients) of available baseline plasma samples, with ESR1 mutations detected in 39.1% of samples (63 of 161).
- We assessed the impact of ESR1 mutations on outcome in patients randomly assigned to receive exemestane ($n = 57$) versus fulvestrant-containing ($n = 104$) regimens.
- For patients with ESR1 mutant ctDNA, the median PFS was 2.6 months (95% CI, 2.4 to 6.2) for patients given exemestane and 5.7 months (95% CI, 3.0 to 8.5) for those given fulvestrant.
- The interaction test between treatment allocation and ESR1 mutation status was $P = .07$.

![FIGURE 2](image-url) Progression-free survival (PFS) in SoFEA by ESR1 mutation status. (A) PFS of patients with ESR1 mutant cancers who received exemestane or a fulvestrant-containing regimen. (B) PFS of patients without detected ESR1 mutation who received exemestane or a fulvestrant-containing regimen.

HR, hazard ratio.
survival in patients treated with exemestane were consistent with the PFS analysis.

Impact of ESR1 mutation on sensitivity to palbociclib in PALOMA3
In PALOMA3, ESR1 mutation status was successfully analyzed in 100% of available samples (360 of 360, representing 69.1% of all patients), with ESR1 mutations detected in 25.3% of patients (91 of 360).

For patients with ESR1 mutant ctDNA, the median PFS was 9.4 months (95% CI, 5.3 to 11.1) for those taking fulvestrant and palbociclib, compared with 3.6 months (95% CI, 2.0 to 5.5) for those taking fulvestrant and placebo (Figure 3A; HR, 0.49; 95% CI, 0.29 to 0.80; P = .002). For ESR1 wild-type patients, the PFS was 9.5 months (95% CI, 5.3 to 11.1) for those taking fulvestrant and placebo (Figure 3B; HR, 0.49; 95% CI, 0.30 to 0.71; P < .001). The benefit of palbociclib was therefore seen despite ESR1 mutation status (interaction P = .74). The confirmed objective response rates were not significantly different between the ESR1 mutant and ESR1 wild-type patients, but a negative impact of ESR1 mutations on response to fulvestrant and palbociclib cannot be excluded.

Clinical and pathologic associations of ESR1 mutations
With different ESR1 mutation rates observed between the studies, we investigated which clinical and pathologic features were associated with ESR1 mutations. SoFEA recruited a relatively homogenous population of postmenopausal women with prior sensitivity to an AI, and there were no significant differences in baseline characteristics between patients with and without ESR1 mutations. A more diverse population of patients whose disease had progressed after receiving prior endocrine therapy was recruited for PALOMA3. As in a prior retrospective study,7 ESR1 mutations were almost exclusively found in patients with prior AI exposure with or without tamoxifen and were rare in patients with prior tamoxifen exposure only (Table 1; 28.9% [90 of 311] vs 2.0% [one of 49], respectively; P < .001). ESR1 mutation was associated with sensitivity to prior endocrine
### TABLE 1 - Baseline characteristics of *ESR1* mutant patients versus *ESR1* wild-type patients in PALOMA3

<table>
<thead>
<tr>
<th></th>
<th><em>ESR1</em> mutant (n = 91)</th>
<th><em>ESR1</em> wild-type (n = 269)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at random assignment, years (IQR)</td>
<td>59 (50, 66)</td>
<td>56 (48, 65)</td>
<td>.2</td>
</tr>
<tr>
<td>Hormone receptor status, no. (%)*</td>
<td></td>
<td></td>
<td>.016</td>
</tr>
<tr>
<td>ER-positive/PR-positive</td>
<td>69 (75.8)</td>
<td>173 (64.3)</td>
<td></td>
</tr>
<tr>
<td>ER-positive/PR-negative</td>
<td>17 (18.7)</td>
<td>87 (32.3)</td>
<td></td>
</tr>
<tr>
<td>Disease-free interval (months), no. (%)†</td>
<td></td>
<td></td>
<td>.22</td>
</tr>
<tr>
<td>≤24</td>
<td>6 (11.3)</td>
<td>38 (19.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;24</td>
<td>47 (88.7)</td>
<td>157 (80.5)</td>
<td></td>
</tr>
<tr>
<td>Menopausal status, no. (%)</td>
<td></td>
<td></td>
<td>.07</td>
</tr>
<tr>
<td>Premenopausal/perimenopausal</td>
<td>12 (13.2)</td>
<td>61 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>79 (86.8)</td>
<td>208 (77.3)</td>
<td></td>
</tr>
<tr>
<td>Sensitivity to prior endocrine treatment, no. (%)</td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Yes</td>
<td>85 (93.4)</td>
<td>200 (74.4)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6 (6.6)</td>
<td>69 (25.7)</td>
<td></td>
</tr>
<tr>
<td>Visceral metastases, no. (%)</td>
<td></td>
<td></td>
<td>.11</td>
</tr>
<tr>
<td>Yes</td>
<td>62 (68.1)</td>
<td>157 (58.4)</td>
<td></td>
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<tr>
<td>No</td>
<td>29 (31.9)</td>
<td>112 (41.6)</td>
<td></td>
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<tr>
<td>Bone metastases, no. (%)</td>
<td></td>
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<td>.001</td>
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<tr>
<td>Yes</td>
<td>80 (87.9)</td>
<td>191 (71.0)</td>
<td></td>
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<tr>
<td>No</td>
<td>11 (12.1)</td>
<td>79 (29.0)</td>
<td></td>
</tr>
<tr>
<td>Soft tissue/nodal metastases, no. (%)</td>
<td></td>
<td></td>
<td>.04</td>
</tr>
<tr>
<td>Yes</td>
<td>28 (30.8)</td>
<td>118 (43.9)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63 (69.2)</td>
<td>151 (56.1)</td>
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<tr>
<td>Prior endocrine therapies, no. (%)</td>
<td></td>
<td></td>
<td>&lt;.001</td>
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<tr>
<td>Tamoxifen only</td>
<td>1 (1.1)</td>
<td>48 (17.8)</td>
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<tr>
<td>AI only</td>
<td>41 (45.1)</td>
<td>103 (38.3)</td>
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<td>AI and tamoxifen</td>
<td>49 (53.9)</td>
<td>118 (43.9)</td>
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<td>Prior chemotherapy, no. (%)</td>
<td></td>
<td></td>
<td>.05</td>
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<tr>
<td>Neoadjuvant/adjuvant</td>
<td>32 (35.2)</td>
<td>123 (45.7)</td>
<td></td>
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<tr>
<td>Metastatic ± adjuvant</td>
<td>24 (26.4)</td>
<td>79 (29.4)</td>
<td></td>
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<tr>
<td>None</td>
<td>35 (38.5)</td>
<td>67 (24.9)</td>
<td></td>
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<tr>
<td>Prior lines of therapy for metastatic disease, no. (%)</td>
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<td></td>
<td>.01</td>
</tr>
<tr>
<td>0</td>
<td>14 (15.4)</td>
<td>67 (24.9)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>41 (45.1)</td>
<td>122 (45.4)</td>
<td></td>
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<tr>
<td>2</td>
<td>22 (24.2)</td>
<td>63 (23.4)</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>14 (15.4)</td>
<td>17 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Disease sites, no. (%)</td>
<td></td>
<td></td>
<td>.74</td>
</tr>
<tr>
<td>1</td>
<td>32 (35.2)</td>
<td>81 (31.1)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21 (23.1)</td>
<td>80 (29.7)</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>38 (41.8)</td>
<td>108 (40.2)</td>
<td></td>
</tr>
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</table>

To correct for multiple comparisons, associations with baseline characteristics were considered significant at P < .01.

*Local testing, analysis omits five *ESR1* mutant patients and nine *ESR1* wild-type patients classified as either ER-negative/PR-positive or ER/PR unknown.

Denominator refers to patients who received adjuvant therapy (n = 53 patients with *ESR1* mutation, n = 195 patients with *ESR1* wild type).

AI, aromatase inhibitor; ER, estrogen receptor; IQR, interquartile range; PR, progesterone receptor.
therapy (sensitive to prior endocrine therapy, 29.8% [85 of 285] vs. resistant, 8.0% [six of 75]; P < .001). 

$ESR1$ mutation was significantly associated with bone metastases ($P = .001$) and prior lines of therapy for metastatic disease ($P = .01$; Table 1). In multivariable analysis, $ESR1$ mutation status remained significantly associated with exposure to an AI and sensitivity to endocrine therapy, and with bone or visceral disease.

Impact of individual mutations and clonality

In PALOMA3 overall, patients with $ESR1$ mutations had marginal statistical significance toward worse PFS compared with $ESR1$ wild type in both univariate analysis (HR, 1.46; 95% CI, 1.06 to 2.02; $P = .02$) and multivariable analysis (HR, 1.49; 95% CI, 1.07 to 2.08; $P = .02$). In both studies, there was a predominance of mutations in D538G, Y537N, Y537S, and E380Q (Table 2). Mutations were polyclonal in 49.1% of $ESR1$ mutant samples (27 of 55) in SoFEA and in 28.6% (26 of 91) in PALOMA 3. The lower rate of mutations and polyclonality in PALOMA3 likely reflects the inclusion of patients with tamoxifen exposure and disease with intrinsic resistance to prior endocrine therapy. In vitro, different $ESR1$ mutations show varied sensitivity to fulvestrant, and we explored the impact of individual mutations on outcome with fulvestrant using a post hoc combined analysis of fulvestrant groups in both studies (fulvestrant-containing in SoFEA and fulvestrant plus placebo in PALOMA3, n = 224). No significant difference was observed in PFS for the individual mutations D538G, E380Q, or Y537S, or for patients with polyclonal versus monoclonal mutations (all $P > .1$), although these analyses are limited by their exploratory nature and sample size.

**Discussion**

Results from this prospective-retrospective study on archival samples demonstrate that plasma DNA analysis has potential clinical utility for patients with advanced ER-positive breast cancer that has progressed after prior AI therapy. In patients from the SoFEA trial, the detection of $ESR1$ mutations in plasma DNA predicted relative resistance to exemestane and relative sensitivity to fulvestrant. In contrast, patients without $ESR1$ mutations detected may derive further benefit from exemestane, as well as fulvestrant. Patients with $ESR1$ mutant cancers have a poor prognosis, and the combination of palbociclib and fulvestrant 500 mg...
plasma. This finding will open up large traditionally seen as suboptimally processed for accurate ctDNA analysis in what are DNA from white blood cell lysis, allowing the release of contaminating free germline is robust for mutation detection, despite merase chain reaction (PCR). This technique used for ctDNA analysis using digital polymerase chain reaction (PCR). This provides the first evidence of potential clinical utility for the use of ESR1 plasma DNA analysis in selecting the most appropriate endocrine therapy. It should be noted that although we assessed seven different ESR1 mutations, there may be other mutations or aberrations in ESR1, such as amplification or rearrangement, that could also contribute to AI resistance.27

Our results suggest that ESR1 mutant cancers show selective sensitivity to fulvestrant, a drug that degrades the ER, but overall with modestly worse PFS than wild-type cancers. This is consistent with the finding that in vitro hotspot mutations in the ligand-binding domain partially inhibit fulvestrant binding.10 More potent receptor degraders may have the potential to further improve with fulvestrant in ESR1 mutant cancers, and a number of such therapies are in early clinical development. Our data confirm laboratory findings that ESR1 mutant cancers continue to drive cell cycle progression through cyclin D1 activation of CDK4/6 and that CDK4/6 inhibition remains a highly active therapeutic approach in ESR1 mutant cancer when combined with fulvestrant that at least partially blocks mutant ER function.

Our study has a number of important limitations. The biologic analysis was retrospective for both studies, although to mitigate

**Key points**

- Our results demonstrate that archival plasma samples collected in EDTA with substantially delayed processing can be used for ctDNA analysis using digital polymerase chain reaction (PCR).
- ESR1 mutations are a rare cause of intrinsic primary endocrine resistance and are observed in advanced ER-positive breast cancer during the development of acquired secondary resistance to AI therapy.
- Results are consistent with our prior retrospective analysis that showed, in a single-center retrospective series, that patients with ESR1 mutations had a poor PFS on subsequent AI-based therapy.7 Here, in a prospective-retrospective analysis of SoFEA, we observed that patients with ESR1 mutations detected in plasma had poor PFS on further AI therapy, specifically, exemestane, but relatively improved PFS when treated with fulvestrant. This provides the first evidence of potential clinical utility for the use of ESR1 plasma DNA analysis in selecting the most appropriate endocrine therapy.26 It should be noted that although we assessed seven different ESR1 mutations, there may be other mutations or aberrations in ESR1, such as amplification or rearrangement, that could also contribute to AI resistance.27

Our study has a number of important limitations. The biologic analysis was retrospective for both studies, although to mitigate
Syllabus

Key points

- ESR1 mutations are found at high frequency in patients who progress after taking prior AIs and can be analyzed relatively simply and cheaply with digital PCR.
- Our data suggest ESR1 mutation analysis may have clinical utility in directing further endocrine therapy, although further confirmatory studies are required.
- Our results demonstrate that ESR1 mutant and wild-type cancers seem to be distinct subtypes of breast cancer that differ in response to standard endocrine therapies.

References

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