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


See accompanying editorial on page 2950

ABSTRACT

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 0.76; P < .001).

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

J Clin Oncol 34:2961-2968. © 2016 by American Society of Clinical Oncology

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 cancers previously treated with aromatase inhibitors (AIs),7–9 implying evolution


Processed as a Rapid Communication manuscript.

Supported by The Royal Marsden Charity—Le Cire Fund; the Medical Research Council, Grant No. MR/N002121/1; Breast Cancer Now with support from the Mary-Jean Mitchell Green Foundation; Cancer Research UK, Grant No. C30746/A16642; and Pfizer. The SoFEA trial was funded by Cancer Research UK, Grant No. C149/1/A10682, reference Nos. CRUK/E03021 and CRUK/ 09/007, and an educational grant from AstraZeneca. The Institute of Cancer Research Clinical Trials and Statistics Unit receives grant funding from Cancer Research UK, Grant No. C1491/ A15685. We acknowledge National Institute for Health Research funding to the Royal Marsden and Institute of Cancer Research Biomedical Research Centre.

C.F. and B.O.L. contributed equally to this work.

Authors’ disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Clinical trial information: NCT01942135, NCT02063422.

Corresponding author: Nicholas C. Turner, The Royal Marsden and Institute of Cancer Research, 237 Fulham Rd, London, SW3 6JB, UK; email: nicholas.turner@kcr.ac.uk.

© 2016 by American Society of Clinical Oncology

0732-183X/16/3425w-2961w/$20.00
DOI: 10.1200/JCO.2016.67.3061

See accompanying editorial on page 2950

ABSTRACT

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 0.76; 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.

J Clin Oncol 34:2961-2968. © 2016 by American Society of Clinical Oncology

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 cancers previously treated with aromatase inhibitors (AIs),7–9 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.8-11 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.7,12-15

The most effective treatment of $ESR1$ mutant breast cancer is uncertain.8,9 In a retrospective, single-center analysis, we have demonstrated resistance to subsequent AI-based therapy in patients with $ESR1$ mutations in plasma.7 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.8 Palbociclib, a CDK4/6 inhibitor, has demonstrated substantial clinical activity in combination with both fulvestrant and AIs.16,17 CDK4/6 inhibition resensitizes cells with in vitro-derived resistance to endocrine therapy,18 and $ESR1$ mutant models are sensitive to combinations of selective ER degraders with palbociclib.19

In this study, we used ctDNA analysis to identify $ESR1$ mutant cancers and assess the impact of mutations on the efficacy of current therapies. Baseline plasma samples were drawn from two

---

Fig 1. CONSORT diagram demonstrating samples analyzed in the (A) SoFEA and (B) PALOMA3 trials. *Only samples after January 2, 2008, were available after a fire at The Royal Marsden Hospital.
Randomized phase III studies that spanned the development of standard endocrine-based therapy for breast cancer progressing after prior endocrine therapy.\textsuperscript{17,20} The SoFEA (Study of Faslodex Versus Exemestane With or Without Arimidex) trial showed no significant difference in its primary end point of progression-free survival (PFS) between fulvestrant 250 mg, fulvestrant 500 mg plus anastrozole, and exemestane in a population previously sensitive to AIs.\textsuperscript{20} The PALOMA3 (Palbociclib Combined With Fulvestrant in Hormone Receptor–Positive HER2-Negative Metastatic Breast Cancer After Endocrine Failure) study demonstrated that palbociclib improves PFS (its primary end point) when added to fulvestrant 500 mg in patients with progression after receiving prior endocrine therapy.\textsuperscript{17} From our prior retrospective study,\textsuperscript{7} we hypothesized that \textit{ESR1} mutant patients would have a poor prognosis when treated with exemestane and that this prognosis would be improved with fulvestrant (samples from SoFEA), with additional improvement with palbociclib (samples from PALOMA3).

\section*{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.\textsuperscript{20} 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 (Fig 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 (Data Supplement). Written informed consent was obtained from all participants, and \textit{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.\textsuperscript{17} 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 the PALOMA3 trial (Fig 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 (Data Supplement). Written informed consent was obtained from all participants.

\section*{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 $-80^\circ$C until DNA extraction. DNA extraction was performed using the QIamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

\section*{RESULTS}

\subsection*{Impact of ESR1 Mutation on Sensitivity to Endocrine Therapies in SoFEA}

In SoFEA, \textit{ESR1} mutation status was successfully analyzed in 99.4% (161 of 162) of all patients.
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 (Fig 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 (Fig 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 (Data Supplement; 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 in patients treated with exemestane were consistent with the PFS analysis (Data Supplement).

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 (Fig 3A; HR, 0.43; 95% CI, 0.25 to 0.74; P = .002). For ESR1 wild-type patients, the PFS was 9.5 months (95% CI, 9.2 to not estimable) for those taking fulvestrant and palbociclib and 5.4 months (95% CI, 3.5 to 7.4) for those taking fulvestrant and placebo (Fig 3B; HR, 0.49; 95% CI, 0.35 to 0.70; P < .001). The benefit from 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 (Data Supplement), 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 (Data Supplement).

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, 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 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 (Data Supplement).

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 = .012) and multivariable analysis (HR, 1.43; 95% CI, 1.03 to 1.99; P = .033). The HRs were most statistically significant in patients with prior tamoxifen exposure only (HR, 2.02; 95% CI, 1.31 to 3.13; P = .001) and prior lines of therapy for metastatic disease (HR, 1.59; 95% CI, 1.07 to 2.36; P = .02).

Supplement).
to 2.02; \( P = .02 \); Data Supplement) and multivariable analysis (HR, 1.49; 95% CI, 1.07 to 2.08; \( P = .02 \); Data Supplement). In both studies, there was a predominance of mutations in D538G, Y357N, Y357S, 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 (Data Supplement). The lower rate of mutations and polyclonality in PALOMA 3 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 PALOMA 3, \( n = 224 \)). No significant difference was observed in PFS for the individual mutations D538G, E380Q, or Y357S, or for patients with polyclonal versus monoclonal mutations (all \( P > .1 \); Data Supplement), 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 appeared to be equally effective in patients with or without \( ESR1 \) mutations (interaction test \( P = .74 \)), although further studies are required to confirm the efficacy of CDK4/6 inhibitors in \( ESR1 \) mutant cancer.

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). This technique is robust for mutation detection, despite the release of contaminating free germline DNA from white blood cell lysis, allowing for accurate ctDNA analysis in what are traditionally seen as suboptimally processed samples. This finding will open up large archival plasma sets linked to phase III trials for ctDNA analysis using digital PCR. Samples were collected in EDTA tubes, which chelate calcium and inhibit blood DNases, and it is unknown whether the findings apply to samples collected with alternative anticoagulants or other methods of \( ESR1 \) detection. The finding on multivariable analysis that detection of \( ESR1 \) mutations is associated with bone and visceral disease may suggest limited detection in patients with nodal or locoregional recurrence only, and this should be considered in future studies.

\( ESR1 \) 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 (Table 1). This is consistent with the higher rate of \( ESR1 \) mutations observed in SoFEA, where recruited patients had all demonstrated previous clinical benefit from an AI. As a consequence, there were no clinical-pathologic associations of \( ESR1 \) mutations in the SoFEA cohort (Data Supplement) that could confound the observed predictive effects (Fig 2). The prior hormone sensitivity of patients in SoFEA may contribute to the residual sensitivity of \( ESR1 \) wild-type cancers to exemestane (Fig 2B; Data Supplement), suggesting that exemestane may be still active in tumors that have acquired resistance to nonsteroidal AIs without selection of an \( ESR1 \) mutation.
These 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. 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. 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.

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. 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 bias, prespecified statistical analysis plans were developed. A relatively small number of baseline samples were available from the SoFEA trial (162), which limits the statistical power to detect important interactions and differences, such as the interaction test between ESR1 mutation and relative sensitivity to fulvestrant over time.

| Table 1. Baseline Characteristics of ESR1 Mutant Patients Versus ESR1 Wild-Type Patients in PALOMA3 |
|----------------------------------|----------------------------------|----------------------------------|
| ESR1 Mutant (n = 91) | ESR1 Wild Type (n = 269) | P   |
| Median age at random assignment, years (IQR) | 59 (50, 66) | 56 (48, 65) | .2  |
| Hormone receptor status, No. (%)* | | | |
| ER-positive/PR-positive | 69 (75.8) | 173 (64.3) | .016 |
| ER-positive/PR-negative | 17 (18.7) | 87 (32.3) | |
| Disease-free interval (months), No. (%)† | | | .22 |
| ≤ 24 | 6 (11.3) | 38 (19.5) | |
| > 24 | 47 (88.7) | 157 (80.5) | |
| Menopausal status, No. (%) | | | .07 |
| Premenopausal/perimenopausal | 12 (13.2) | 61 (22.7) | |
| Postmenopausal | 79 (86.8) | 206 (77.3) | |
| Sensitivity to prior endocrine treatment, No. (%) | | | < .001 |
| Yes | 85 (93.4) | 200 (74.4) | |
| No | 6 (6.6) | 69 (25.7) | |
| Visceral metastases, No. (%) | | | .11 |
| Yes | 62 (68.1) | 157 (58.4) | |
| No | 29 (31.9) | 112 (41.6) | |
| Bone metastases, No. (%) | | | .001 |
| Yes | 80 (87.9) | 191 (71.0) | |
| No | 11 (12.1) | 79 (29.0) | |
| Soft tissue/nodal metastases, No. (%) | | | .04 |
| Yes | 28 (30.8) | 118 (43.9) | |
| No | 63 (69.2) | 151 (56.1) | |
| Prior endocrine therapies, No. (%) | | | < .001 |
| Tamoxifen only | 1 (1.1) | 48 (17.8) | |
| AI only | 41 (45.1) | 103 (38.3) | |
| AI and tamoxifen | 49 (53.9) | 118 (43.9) | |
| Prior chemotherapy, No. (%) | | | .05 |
| Neoadjuvant/adjuvant | 32 (35.2) | 123 (45.7) | |
| Metastatic ± adjuvant | 24 (26.4) | 79 (29.4) | |
| None | 35 (38.5) | 67 (24.9) | |
| Prior lines of therapy for metastatic disease, No. (%) | | | .01 |
| 0 | 14 (15.4) | 67 (24.9) | |
| 1 | 41 (45.1) | 122 (45.4) | |
| 2 | 22 (24.2) | 63 (23.4) | |
| 3+ | 14 (15.4) | 17 (6.3) | |
| Disease sites, No. (%) | | | .74 |
| 1 | 32 (35.2) | 81 (3.1) | |
| 2 | 21 (23.1) | 80 (29.7) | |
| 3+ | 38 (41.8) | 108 (40.2) | |

NOTE. To correct for multiple comparisons, associations with baseline characteristics were considered significant at P < .01. Abbreviations: AI, aromatase inhibitor; ER, estrogen receptor; IQR, interquartile range; PR, progesterone receptor.

*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).
exemestane ($P = .07$). Further confirmatory studies are required before it could be concluded that plasma ESR1 mutation analysis may be used to guide treatment. Although samples were collected in multiple sites, analysis was centralized in a single laboratory. No assessment of interlaboratory agreement has yet been conducted with the assays, and this would be required before ESR1 digital PCR can be used in clinical decision making. The SoFEA trial recruited only patients with sensitivity to prior AIs, and it is unknown whether the results would also apply to patients who were not sensitive to prior AIs. The exploratory analyses in this report are hypothesis generating and will require confirmation in future studies.

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 that 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. Future clinical trials in advanced breast cancer might consider using plasma DNA analysis to optimize endocrine therapy choice according to ESR1 mutation status.

### REFERENCES


### AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

### AUTHOR CONTRIBUTIONS


Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

Table 2. Frequency of Specific ESR1 Mutations in the SoFEA and PALOMA3 Cohorts

<table>
<thead>
<tr>
<th>ESR1 Mutation</th>
<th>No. of Mutations Observed in Cohort</th>
<th>% of SoFEA ESR1 Mutant (n = 63)</th>
<th>% of All SoFEA Cohort (n = 161)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D538G</td>
<td>29</td>
<td>46.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Y537N</td>
<td>23</td>
<td>36.5</td>
<td>14.3</td>
</tr>
<tr>
<td>Y537S</td>
<td>16</td>
<td>25.4</td>
<td>9.9</td>
</tr>
<tr>
<td>E380Q</td>
<td>6</td>
<td>9.5</td>
<td>3.7</td>
</tr>
<tr>
<td>T385P</td>
<td>6</td>
<td>9.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Y537C</td>
<td>3</td>
<td>4.8</td>
<td>1.9</td>
</tr>
<tr>
<td>L536R</td>
<td>2</td>
<td>3.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ESR1 Mutation</th>
<th>No. of Mutations Observed in Cohort</th>
<th>% of PALOMA ESR1 Mutant (n = 91)</th>
<th>% of All PALOMA Cohort (n = 360)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D538G</td>
<td>51</td>
<td>56.0</td>
<td>14.2</td>
</tr>
<tr>
<td>Y537N</td>
<td>14</td>
<td>15.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Y537S</td>
<td>23</td>
<td>25.3</td>
<td>6.4</td>
</tr>
<tr>
<td>E380Q</td>
<td>22</td>
<td>24.2</td>
<td>6.1</td>
</tr>
<tr>
<td>T385P</td>
<td>4</td>
<td>4.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Y537C</td>
<td>5</td>
<td>5.5</td>
<td>1.4</td>
</tr>
<tr>
<td>L536R</td>
<td>1</td>
<td>1.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Plasma ESR1 Mutations and Advanced ER-Positive Breast Cancer

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

Charlotte Fribbens
No relationship to disclose

Ben O’Leary
Research Funding: Pfizer

Lucy Kilburn
No relationship to disclose

Sarah Hrebien
No relationship to disclose

Isaac Garcia-Murillas
No relationship to disclose

Matthew Beaney
Research Funding: Randox Laboratories (I)

Massimo Cristofanilli
Honoraria: Agendia
Company: Dompe Farmaceutici, Celgene, Pfizer
Consulting or Advisory Role: Dompe Farmaceutici, Cynvenio Biosystems, Newomics

Fabrice Andre
Research Funding: AstraZeneca (Inst), Novartis (Inst), Pfizer (Inst), Eli Lilly (Inst)

Sherene Loi
Research Funding: Genentech (Inst), Pfizer (Inst), Novartis (Inst), Merck (Inst)
Patents, Royalties, Other Intellectual Property: PI3K pathway gene signature granted by the European and US patent offices

Sibylle Loibl
Research Funding: Novartis (Inst), Pfizer (Inst) Genentech (Inst)

John Jiang
Employment: Pfizer
Stock or Other Ownership: Pfizer

Cynthia Huang Bartlett
Employment: Pfizer
Stock or Other Ownership: Pfizer

Maria Koehler
Employment: Pfizer
Stock or Other Ownership: Pfizer

Mitch Dowsett
Consulting or Advisory Role: Radius
Speakers’ Bureau: AstraZeneca, Myriad Genetics
Research Funding: AstraZeneca (Inst)

Judith M. Bliss
Research Funding: AstraZeneca (Inst), Pfizer (Inst), Janssen Cilag (Inst), Novartis (Inst), Roche (Inst), Clovis Oncology (Inst)

Stephen R.D. Johnston
Consulting or Advisory Role: Eli Lilly, AstraZeneca, Novartis,
Speakers’ Bureau: GlaxoSmithKline, Roche
Research Funding: Pfizer (Inst)

Nicholas C. Turner
Consulting or Advisory Role: Roche, Pfizer, Novartis, AstraZeneca
Research Funding: Pfizer (Inst), Roche (Inst), AstraZeneca
We thank the patients who participated in the SoFEA and PALOMA3 studies, along with the investigators, study nurses, and site staff who supported the trial and collected plasma samples. We acknowledge the staff of the department of Academic Biochemistry at the Royal Marsden, who processed samples in SoFEA.