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

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ABSTRACT

Purpose: *ESR1* mutations are acquired frequently in hormone receptor–positive metastatic breast cancer after prior aromatase inhibitors. We assessed the clinical utility of baseline *ESR1* circulating tumor DNA (ctDNA) analysis in the two phase III randomized trials of fulvestrant versus exemestane.

Experimental Design: The phase III EFECT and SoFEA trials randomized patients with hormone receptor–positive metastatic breast cancer who had progressed on prior nonsteroidal aromatase inhibitor therapy, between fulvestrant 250 mg and exemestane. Baseline serum samples from 227 patients in EFECT, and baseline plasma from 161 patients in SoFEA, were analyzed for *ESR1* mutations by digital PCR. The primary objectives were to assess the impact of *ESR1* mutation status on progression-free (PFS) and overall survival (OS) in a combined analysis of both studies.

Results: *ESR1* mutations were detected in 30% (151/383) baseline samples. In patients with *ESR1* mutation detected, PFS was 2.4 months [95% confidence interval (CI), 2.0–2.6] on exemestane and 3.9 months (95% CI, 3.0–6.0) on fulvestrant [hazard ratio (HR), 0.59; 95% CI, 0.39–0.89; $P = 0.01$). In patients without *ESR1* mutations detected, PFS was 4.8 months (95% CI, 3.7–6.2) on exemestane and 4.1 months (95% CI, 3.6–5.5) on fulvestrant (HR, 1.05; 95% CI, 0.81–1.37; $P = 0.69$). There was an interaction between *ESR1* mutation and treatment ($P = 0.02$). Patients with *ESR1* mutation detected had 1-year OS of 62% (95% CI, 45%–75%) on exemestane and 80% (95% CI, 68%–87%) on fulvestrant ($P = 0.04$; restricted mean survival analysis). Patients without *ESR1* mutations detected had 1-year OS of 79% (95% CI, 71%–85%) on exemestane and 81% (95% CI, 74%–87%) on fulvestrant ($P = 0.69$).

Conclusions: Detection of *ESR1* mutations in baseline ctDNA is associated with inferior PFS and OS in patients treated with exemestane versus fulvestrant.

Introduction

Mutations in the estrogen receptor gene (*ESR1*) are acquired frequently in metastatic hormone receptor–positive breast cancer (1, 2). Mutations are selected in the cancer as a mechanism of clinical acquired resistance to prior aromatase inhibitor therapy, acquired relatively rarely through tamoxifen (3, 4). *ESR1* mutations are acquired most frequently when aromatase inhibitors are used to treat advanced breast cancer (5), are more frequently selected in cancers that progress after sensitivity to prior aromatase inhibitor therapy, and are relatively rare in patients with intrinsic endocrine resistance (3). This presents challenges in the identification of *ESR1* mutations in standard clinical practice, as although biopsy of a recurring breast cancer is now commonplace, repeat biopsy after initial treatment is rarely performed. Multiple studies have shown that *ESR1* mutations can be identified at high frequency in the plasma, in the circulating tumor DNA (ctDNA), of patients after progression on aromatase inhibitor therapy (3, 6, 7).

Multiple sequential lines of endocrine-based therapy is a standard of care for advanced hormone receptor–positive cancer, especially in patients whose cancer shows sensitivity to prior or first-line hormone therapy (8). Two phase III trials [the soFEA (9) and EFECT (10) studies] investigated the optimal second-line endocrine therapy, randomizing patients progressing on a nonsteroidal aromatase inhibitor between fulvestrant 250 mg and exemestane. In prior analysis, we analyzed *ESR1* mutations in baseline plasma from the SoFEA trial. 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% confidence interval (CI), 0.30–0.92; $P = 0.02$], whereas patients with wild-type *ESR1* had similar PFS after receiving either treatment (HR, 1.07; 95% CI, 0.68 to 1.67; $P = 0.77$; ref. 3). Baseline serum samples were available from EFECT, with no plasma samples available. Serum samples present challenges for ctDNA analysis due to release of lymphocyte DNA during clotting (11), although in prior research we have shown that release of wild-type DNA does not substantially effect results of ctDNA analysis with digital PCR (3).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2020;26:5172–7

doi: 10.1158/1078-0432.CCR-20-0224

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Translational Relevance

In patients previously treated with an aromatase inhibitor, detection of *ESR1* mutations in circulating tumor DNA (ctDNA) analysis identified patients who have worse overall survival if treated with exemestane instead of fulvestrant. For patients with acquired *ESR1* mutations, further aromatase inhibitor therapy is not appropriate, and analysis of ctDNA may be considered when selecting endocrine therapy backbone in patients progressing after prior aromatase inhibitor therapy.

To assess the clinical utility of *ESR1* mutation analysis in ctDNA, we analyzed baseline *ESR1* mutation status in patients entering the EFECT study, and then performed a combined analysis of SoFEA and EFECT to investigate the interaction between *ESR1* mutation status and relative benefit of second-line endocrine therapies, and the impact of *ESR1* mutation status on overall survival (OS) on second-line endocrine therapies.

Materials and Methods

Study designs

The EFECT study was a randomized, placebo-controlled, double-placebo phase III trial conducted in postmenopausal women with advanced hormone receptor–positive breast cancer who had previously progressed on a nonsteroidal aromatase inhibitor. Patients were randomized between fulvestrant 250 mg with a loading dose (500 mg intramuscularly on day 1, followed by 250 mg on days 15 and 29, then every 28 days) or exemestane 25 mg. Baseline serum was available in 227 patients of 693 patients enrolled (33%; **Fig. 1**). The subset of patients with baseline serum available had similar baseline characteristics and outcome to patients without samples (Supplementary Tables S1 and S2).

The SoFEA study was a multicenter, randomized phase III trial in postmenopausal women with advanced, hormone receptor–positive breast cancer who had demonstrated sensitivity to prior nonsteroidal aromatase inhibitors, defined as relapse or progression after taking adjuvant treatment for at least 12 months or as first-line metastatic treatment for at least 6 months (9). Patients were assigned fulvestrant at the same dosing schedule as EFECT plus anastrozole 1 mg, fulvestrant plus placebo, or exemestane 25 mg. Both fulvestrant groups were merged for analysis, and *ESR1* mutations were analyzed in 161 baseline plasma samples as described previously (3).

Both EFECT and SoFEA were approved by ethical or institutional review boards as detailed previously (9, 10), carried out as per the Declaration of Helsinki, and written informed consent was supplied by all participants.

ESR1 mutation analysis

For EFECT, baseline serum samples analysis of *ESR1* mutations was conducted essentially as described previously (3), blinded to clinical results. Multiplex digital PCR was used as the analysis method, a method that is largely unaffected by contamination of the sample with lymphocyte DNA (Supplementary Fig. S1; ref. 3). DNA was extracted from up to 1 mL of baseline samples and analyzed by droplet digital PCR on a QX200 system with two multiplex digital PCR assays. Multiplex 1 included c.1138G.C(E380Q), c.1607T.G(L536R), c.1610A.G(Y537C), and c.1613A.G(D538G; dHsaMDXE91450042); multiplex 2 included c.1387T.C(S463P), c.1609T.A(Y537N), and c.1610A.C (Y537S; dHsaMDXE65719815).

Statistical analysis

The combined analysis had two primary objectives: to assess whether there was an interaction on PFS between *ESR1* mutation status and treatment randomization between exemestane and fulvestrant, and to assess OS in patients with baseline *ESR1* mutation detection. The primary endpoint of EFECT was time to progression (defined as objective disease progression or death) and the primary endpoint of SoFEA was PFS (defined as objective disease progression, second primary cancer necessitating a change in systemic treatment, or death), both using RECIST 1.0. The primary endpoints of the combined analysis were PFS (defined as in the original trial) and OS, in the subset of patients with successful mutation analysis and with treatment

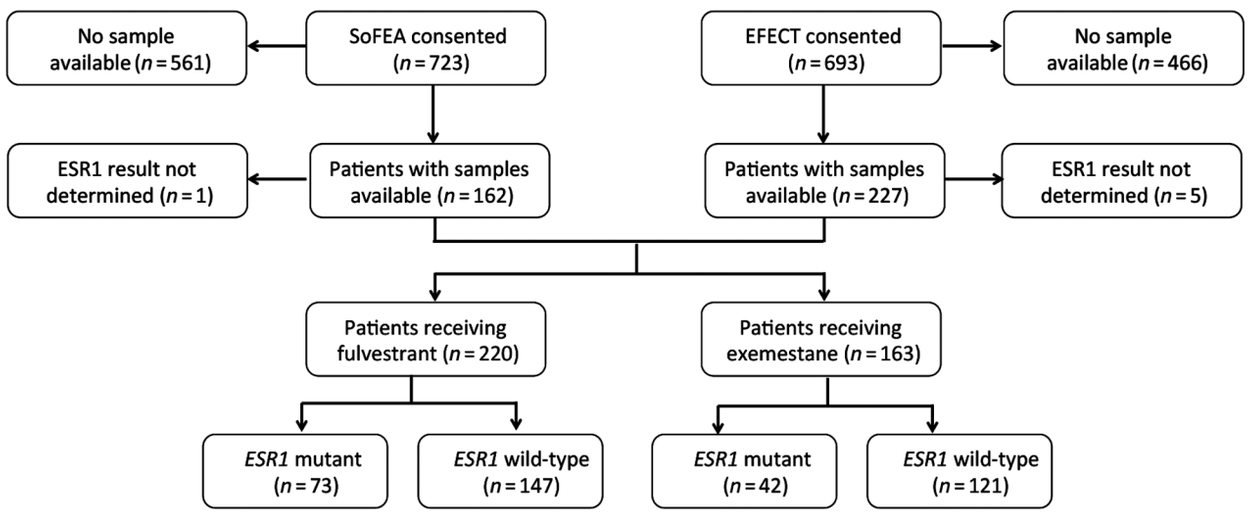

Figure 1.
CONSORT diagram of EFECT and SoFEA combined analysis.

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randomly assigned on an intention-to-treat basis. *ESR1* mutation analysis was retrospective, not conceived in the original study protocols as they predated the discovery of *ESR1* mutations as a frequent mechanism of aromatase inhibitor resistance.

The relationship between baseline *ESR1* mutation status and PFS and OS was assessed with a Cox proportional hazards regression model along with an interaction test to explore differential effects between *ESR1* mutation status and trial treatment where relevant. All analyses were stratified by trial. The proportionality assumption of the Cox models was tested with Schoenfeld residuals. As OS analysis in the *ESR1* mutation–detected group was shown to be nonproportional, a restricted mean survival analysis with a cutoff of 24 months was also used for OS analysis. Secondary analyses included the association between detection of *ESR1* mutations and clinical and pathologic variables. All *P* values were two-sided with a significance level of 0.05. All statistical analyses were performed with Stata (version 13.1; STATA).

Results

ESR1 mutation analysis in EFECT

To enable a combined analysis of EFECT and SoFEA, we first analyzed *ESR1* mutation status in baseline serum samples from EFECT (Supplementary Fig. S2). *ESR1* mutations were successfully analyzed in 98% (222/227) of patients with baseline serum samples, with *ESR1* mutations detected in 23.4% (52/222) of samples. (Supplementary Fig. S3).

ESR1 mutations and PFS in the combined analysis

The combined analysis of SoFEA and EFECT comprised a total of 383 patients with baseline sample *ESR1* ctDNA results (**Fig. 1**; Supplementary Table S3), with 326 PFS events. *ESR1* mutations were detected in 30% (115/383) of baseline samples overall, more frequently in patients with sensitivity to prior aromatase inhibitor as defined by the original trials ($P = 0.02$) and by setting and time on prior aromatase inhibitor therapy ($P = 0.01$; **Table 1**). In patients with an *ESR1* mutation detected, median PFS was 2.4 months (95% CI, 2.0–2.6) on exemestane and 3.9 months (95% CI, 3.0–6.0) on fulvestrant (HR, 0.59; 95% CI, 0.39–0.89; $P = 0.01$). In patients without *ESR1* mutations detected, PFS was 4.8 months (95% CI, 3.7–6.2) on exemestane and 4.1 months (95% CI, 3.6–5.5) on fulvestrant (HR, 1.05; 95% CI, 0.81–1.38; $P = 0.69$; **Fig. 2**). There was a statistically significant interaction between treatment randomization and *ESR1* mutation status ($P_{interaction} = 0.02$).

In a multivariate analysis, *ESR1* mutation status was associated with shorter PFS (HR, 1.96; 95% CI, 1.34–2.86; $P = 0.001$), and an interaction with allocated treatment remained significant ($P = 0.05$; **Table 2**). Older age, bone-only disease, and a period of >5 years from initial diagnosis to study entry were associated with longer PFS (**Table 2**).

Patients with D538G, Y537X, and E380Q/S463P mutations detected in ctDNA had similar PFS improvement with fulvestrant compared with exemestane (Supplementary Figs. S4 and S5). Patients with monoclonal *ESR1* mutations had a median PFS on fulvestrant of 3.6 months (95% CI, 2.7–5.7; $N = 70$) and with polyclonal *ESR1* mutations had a median PFS on fulvestrant of 6.6 months (95% CI, 2.9–11.7; $N = 42$; Supplementary Fig. S6).

ESR1 mutations and objective response in the combined analysis

In patients with *ESR1* mutations detected, objective response rate on fulvestrant was 9.5% (4/42; 95% CI, 2.7–22.6) and on exemestane was 0.0% (0/36; 95% CI, 0–9.7; Fisher exact test, $P = 0.12$). In patients without *ESR1* mutations detected, objective response rate on fulvestrant was 9.6% (9/94; 95% CI, 4.5–17.4) and on exemestane was 8.7% (9/103; 95% CI, 4.1–15.9; Fisher exact test, $P = 1.0$). In patients with *ESR1* mutations detected, clinical benefit rate on fulvestrant was 31.0% (13/42; 95% CI, 17.6–47.1) and on exemestane was 22.2% (8/36; 95% CI, 10.1–39.2; Fisher exact test, $P = 0.45$). In patients without *ESR1* mutations detected, clinical benefit rate on fulvestrant was 37.2% (35/94; 95% CI, 27.5–47.8) and on exemestane was 41.7% (43/103; 95% CI, 32.1–51.9; Fisher exact test, $P = 0.56$).

Table 1. Baseline characteristics associated with *ESR1* mutation detection in baseline ctDNA.

	***ESR1* mutant**	**ESR1 wild-type**
	$N = 115$	$N = 268$
Age at randomization (years); $P = 0.09$	*N* (%)	*N* (%)
<50	11 (9.6)	15 (5.6)
50–64	57 (49.6)	115 (42.9)
65–75	29 (25.2)	92 (34.3)
≥75	18 (15.7)	46 (172)
Hormone receptor status; $P = 0.88$		
ER^+, PgR^+	68 (59.1)	151 (56.3)
ER^+, PgR^-	22 (19.1)	57 (21.3)
ER^+, PgR unknown	22 (19.1)	51 (19.0)
ER^-/unknown, PgR^+	1 (0.9)	5 (1.9)
ER unknown, PgR unknown	2 (1.7)	2 (0.8)
ER^-, PgR^-	0 (0.0)	2 (0.8)
Visceral involvement; $P = 0.31$		
Yes	73 (63.5)	155 (57.8)
No	42 (36.5)	113 (42.2)
Site of disease; $P = 0.13$		
Visceral	73 (63.5)	155 (57.8)
Soft tissue/node	20 (17.4)	73 (27.2)
Bone only	22 (19.1)	39 (14.6)
Unknown	0 (0.0)	1 (0.4)
Time from diagnosis to randomization (years); $P = 0.96$		
<1	0 (0.0)	9 (3.4)
1–2	25 (21.7)	43 (16.0)
3–4	16 (13.9)	42 (15.7)
5+	74 (64.4)	174 (64.9)
NSAI setting and time on NSAI; $P = 0.01$		
Adjuvant	15 (13.0)	47 (17.5)
ABC <1 year	14 (12.2)	63 (23.5)
ABC 1–2 years	37 (32.2)	68 (25.4)
ABC 2+ years	49 (42.6)	87 (32.5)
Unknown	0 (0.0)	3 (1.1)
AI status; $P = 0.02$		
Sensitive	96 (83.5)	194 (72.4)
Resistant	19 (16.5)	74 (27.6)

Note: Aromatase inhibitor (AI) status, sensitivity to prior aromatase inhibitor (sensitive indicating relapse after at least 2 years of adjuvant aromatase inhibitor or response or duration of stable disease lasting at least 24 weeks in the metastatic setting). *P* values from χ^2 test.
Abbreviations: ABC, advanced breast cancer; ER, estrogen receptor; NSAI, nonsteroidal aromatase inhibitor; PgR, progesterone receptor.

ESR1 mutations and adverse short-term OS

We investigated the association between *ESR1* mutation status and OS in the combined analysis, with a total of 204 deaths. In patients without *ESR1* mutations detected, median OS was 23.0 months (95% CI, 19.2–25.6) on exemestane and 25.8 months (95% CI, 22.1–29.9) on

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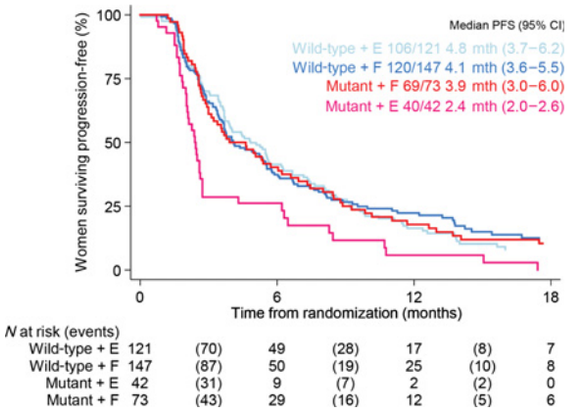

Figure 2.

PFS in the combined analysis of SoFEA and EFECT by *ESR1* mutation status and treatment. Patients with *ESR1* mutation detected: HR, 0.59; 95% CI, 0.39–0.89; $P = 0.01$. Patients without *ESR1* mutation detected: HR, 1.05, 95% CI, 0.81–1.37; $P = 0.69$. Interaction test $P = 0.02$. E, exemestane; F, fulvestrant; mth, month; mutant, *ESR1* mutation detected; wild-type, *ESR1* mutation not detected.

fulvestrant (HR, 0.89; 95% CI, 0.64–1.23; $P = 0.49$; **Fig. 3**). In patients with *ESR1* mutation detected, median OS was 18.0 months (95% CI, 6.8–27.0) on exemestane and 21.2 months (95% CI, 18.3–26.1) on fulvestrant (HR, 0.85; 95% CI, 0.51–1.40; $P = 0.52$). However, Cox proportional hazards assumption was violated for patients with *ESR1* mutations detected (proportionality assumption rho, 0.22; χ^2, 3.86; $P = 0.049$), suggesting nonproportional hazards. We, therefore, repeated the OS analysis utilizing a restricted mean survival model. In patients with *ESR1* mutations detected, patients on exemestane had worse OS compared with those on fulvestrant by restricted mean survival analysis at 24 months (mean difference = −3.3; 95% CI, −6.4 to −0.1; $P = 0.04$), with no difference in the cohort with no detectable *ESR1* mutation (mean difference = −0.8; 95% CI, −2.5 to 0.9; $P = 0.35$). The estimated rates of OS at 1 year in patients with *ESR1* mutation detected was 62% (95% CI, 45%–75%) on exemestane and 80% (95% CI, 68%–87%) on fulvestrant (1-year landmark analysis HR, 0.50; 95% CI, 0.24–1.04; $P = 0.06$; **Fig. 3**). In patients without *ESR1* mutations detected, the 1-year OS was 79% (95% CI, 71%–85%) on exemestane and 81% (95% CI, 74%–87%) on fulvestrant ($P = 0.75$; **Fig. 3**).

Discussion

We conducted a combined analysis of EFECT and SoFEA to investigate the clinical impact of *ESR1* mutation analyzed in ctDNA. Patients with *ESR1* mutations detected had shorter PFS when treated with exemestane therapy, compared with fulvestrant, and also had shorter OS in restricted mean survival analysis. Analysis of OS in patients with *ESR1* mutations suggested nonproportional hazards, suggesting that patients treated with *ESR1*-mutant cancers were at elevated risk of early death if treated with exemestane. Although hormone receptor–positive breast cancer is generally indolent, this may suggest that treatment with an inactive hormone therapy has potential short-term risks for patients, in a subset of patients where *ESR1*-mutant breast cancer may behave more aggressively (12).

In routine clinical practice, exemestane is now frequently given in combination with everolimus (13), potentially limiting the direct translation of these findings to routine practice. However, analysis of *ESR1* mutations in BOLERO2 also suggested adverse outcome for patients with *ESR1* mutations detected in baseline plasma treated with exemestane and everolimus (6). Everolimus has activity when given with multiple different endocrine therapy backbones. Fulvestrant plus everolimus showed substantial activity in the phase II MANTA trial, with 12.2 months PFS (95% CI, 7.5–14.3; ref. 14), and tamoxifen plus everolimus showed substantial activity in the phase II TamRAD trial with 8.6 months PFS (95% CI, 5.9–13.9; ref. 15). In an exploratory

Table 2. Multivariate analysis of PFS in the SoFEA and EFECT combined analysis.

		Hazard ratio (95% CI)	*P*
ESR1 mutation status	Wild-type	1	—
	Mutant	1.96 (1.34–2.86)	0.001
Treatment group	Exemestane	1	—
	Fulvestrant	1.08 (0.82–1.41)	0.6
Age at randomization	<50	1	—
	50–64	0.88 (0.56–1.37)	0.56
	65–75	0.69 (0.44–1.11)	0.13
	≥75	0.55 (0.33–0.91)	0.02
Site of disease	Visceral	1	—
	Soft tissue/node	0.76 (0.58–0.99)	0.04
	Bone only	0.65 (0.46–0.90)	0.01
Time from diagnosis to randomization	<1 year	1	—
	1–2 years	0.58 (0.28–1.19)	0.13
	3–4 years	0.59 (0.28–1.22)	0.15
	5+ years	0.45 (0.22–0.90)	0.02
Hormone receptor status	ER^+, PgR^+	1	—
	ER^+, PgR^-	0.91 (0.69–1.20)	0.5
	ER^+, PgR unknown	0.69 (0.50–0.94)	0.02
	ER^-/unknown, PgR^+	0.51 (0.16–1.62)	0.25
ESR1 mutation status × treatment group (interaction)		0.61 (0.38–1.00)	0.05

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

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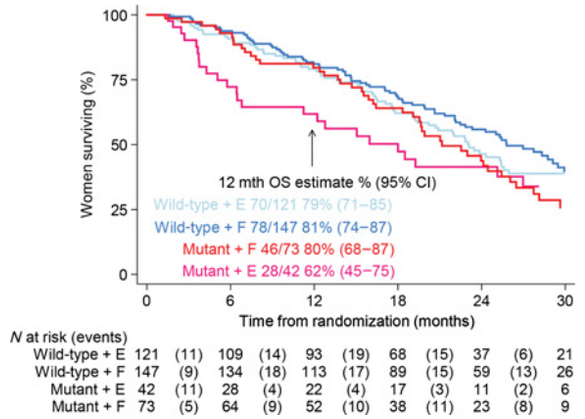

Figure 3.

OS in the combined analysis of SoFEA and EFECT by *ESR1* mutation status and treatment. Patients with *ESR1* mutation detected: restricted mean survival analysis $P = 0.04$. Patients without *ESR1* mutation detected: restricted mean survival analysis $P = 0.69$. E, exemestane; F, fulvestrant; mth, month; mutant, *ESR1* mutation detected; wild-type, *ESR1* not detected.

analysis of the TamRAD study, there was an OS improvement with tamoxifen plus everolimus (HR, 0.45; 95% CI, 0.24–0.84; $P = 0.007$; ref. 15). In contrast, in BOLERO2, exemestane plus everolimus did not demonstrate a statistically significant improvement (HR, 0.89; 95% CI, 0.73–1.10; $P = 0.14$; ref. 16). Although TamRAD was a relatively small phase II study, we speculate that the OS results of exemestane plus everolimus were undermined by inactivity of exemestane in *ESR1*-mutant breast cancer, and that tamoxifen backbone therapy had sufficient activity in *ESR1*-mutant breast cancer to mitigate this effect. Along with the unequivocal preclinical evidence that *ESR1*-mutant cancer is resistant to estrogen deprivation (1, 17), our data suggest that exemestane plus everolimus should be used cautiously in patients with *ESR1* mutations detected in ctDNA, and instead, either fulvestrant or tamoxifen could be considered as alternative backbone endocrine therapy. In exploratory analysis, patients with different *ESR1* mutations detected in ctDNA had similar improvement of outcome on fulvestrant versus exemestane (Supplementary Fig. S5). Although patients with polyclonal *ESR1* mutations in this dataset had numerically improved outcome on fulvestrant compared with monoclonal *ESR1* mutations (Supplementary Fig. S6), this analysis is limited by small numbers, and other datasets have not shown improved outcome of polyclonal versus monoclonal on fulvestrant (18). In the future, further investigation of whether different *ESR1* mutations have differing responsiveness to fulvestrant, or tamoxifen, will be useful in this regard (17).

It is important to emphasize that patients treated with fulvestrant in EFECT and SoFEA were treated with a dose half that is used currently, fulvestrant 250 mg versus fulvestrant 500 mg in the CONFIRM study (19), and this has important implications for interpreting the results of patients with undetected *ESR1* mutations. Although there was no difference between exemestane and fulvestrant (**Figs. 2** and **3**) in the *ESR1*-undetected group, this may simply reflect the lower dose of fulvestrant used; it is likely reasonable to speculate that such patients treated with fulvestrant may have had improved PFS on fulvestrant 500 mg compared with exemestane (19). One other point from our results is that if post–aromatase inhibitor a clinician is considering prescribing fulvestrant 500 mg, there is likely no utility in assessing *ESR1* mutations. Aromatase inhibitor therapy is currently stopped at progression in routine clinical practice. Given the potential efficacy of exemestane in *ESR1* wild-type breast cancer, our findings suggest the possibility that aromatase inhibitor therapy may have activity if continued in subsequent lines of therapy in *ESR1* wild-type tumors. However, prospective trials would be required to validate this hypothesis.

There are limitations to our analysis when considering potential clinical application. Although we provide evidence that *ESR1* mutation ctDNA analysis has predictive and clinical utility, this was with a specific droplet digital PCR *ESR1* mutation ctDNA assay, with analysis conducted in one central laboratory. It is unknown the extent to which these results would be reproduced by different ctDNA assays, and in different laboratories. In prior work, we have shown high reproducibility between the digital PCR assay used in this article and BEAMing digital PCR (20), providing evidence of interassay agreement when conducted in central laboratories. Further research is required on the widespread clinical application of such assays. In EFECT, we analyzed serum samples, which is in general an inferior sample type for ctDNA analysis due to white blood cell lysis releasing contaminating DNA during blood clotting (11). The rate of detection of *ESR1* mutations was not affected by total DNA amounts (Supplementary Fig. S1), suggesting this contamination did not affect the results. The rate of *ESR1* mutations was modestly lower in EFECT serum analysis (23.4%, 52/222) compared with SoFEA plasma analysis (39.1%, 63/161), possibly reflecting lower sensitivity, or reflecting different study populations such as the inclusion of intrinsically endocrine-resistant patients in EFECT, which are known to have a lower incidence of *ESR1* mutations (3). No patients in SoFEA or EFECT had prior exposure to CDK4/6 inhibitors. Patients on a CDK4/6 inhibitor and aromatase inhibitor also acquire *ESR1* mutations, possibly at approximately the same incidence to patients on an aromatase inhibitor alone, although an accurate incidence has not yet been established (21, 22). This suggests our findings will be equally relevant in deciding second-line endocrine therapy backbone now that CDK4/6 inhibitors are a standard of care.

In conclusion, we demonstrate that the detection of *ESR1* mutations in baseline metastatic breast cancer ctDNA analysis predicts lack of benefit from subsequent aromatase inhibitor therapy. Patients with *ESR1* mutations acquired through prior aromatase inhibitor therapy have both adverse PFS and OS when treated with exemestane. Our data provides evidence of clinical utility of ctDNA liquid biopsies in breast cancer, suggesting the potential to improve outcome, to monitor for the presence of *ESR1* mutations in advanced breast cancer, and to aid in selection of the most appropriate subsequent endocrine therapy backbone, if further aromatase inhibitor–based therapy is being considered.

Disclosure of Potential Conflicts of Interest

N.C. Turner reports grants and personal fees from AstraZeneca during the conduct of the study; personal fees from Bristol-Myers Squibb, Lilly, Merck Sharpe & Dohme, and Novartis, grants and personal fees from Pfizer, Roche/Genentech, Tesaro, and Bicycle Therapeutics, grants from BioRad and Clovis, and non-financial support from Guardant Health outside the submitted work. J.F.R. Roberston reports grants from AstraZeneca (investigator in the EFECT study) during the conduct of the study; personal fees from AstraZeneca (honoraria from advisory boards and lectures), other from AstraZeneca (expert testimony), grants from AstraZeneca (chief investigator of FIRST and FALCON trials), and other from University of Nottingham (member of DSM of SoFEA trial) outside the submitted work. M. Piccart reports grants and personal fees from Pfizer, AstraZeneca, and Novartis outside the submitted work. G. Schiavon reports other from AstraZeneca (employee) during the conduct of the study. J.M. Bliss reports grants, non-financial support, and other from AstraZeneca (supply of study drug, database and financial support) and non-financial

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support from Cancer Research UK (support with analysis work) during the conduct of the study; grants and non-financial support from AstraZeneca, Merck Sharpe & Dohme, Puma Biotechnology, Clovis Oncology, Pfizer, Janssen Cilag, and Novartis (previously GSK) outside the submitted work, and grants from Medivation. M. Dowsett reports personal fees from NanoString (lecture fees), Myriad (lecture fees), and Radius (scientific advisory board) outside the submitted work. S.R.D. Johnston reports grants and personal fees from AstraZeneca (speaker honoraria/research funding to institution) during the conduct of the study; grants and personal fees from Pfizer (consultancy/speaker honoraria/research funding to institution), and Eli Lilly (consultancy/research funding to institution), and personal fees from Novartis (consultancy) and Eisai (consultancy) outside the submitted work. S.K. Chia reports grants from AstraZeneca (for conduct of the EFECT trial) and personal fees from AstraZeneca (participation of the advisory board) during the conduct of the study; grants and personal fees from Novartis (participation in advisory board and conduct of clinical trials), Hoffmann LaRoche (participation in advisory board and conduct of clinical trials), Pfizer (participation in advisory board and conduct of clinical trials), and Merck (participation in advisory board and conduct of clinical trials) outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

N.C. Turner: Conceptualization, supervision, funding acquisition, writing-original draft, writing-review and editing. **C. Swift:** Formal analysis, investigation, writing-review and editing. **L. Kilburn:** Formal analysis, validation, writing-review and editing. **C. Fribbens:** Investigation, writing-review and editing. **M. Beaney:** Investigation, writing-review and editing. **I. Garcia-Murillas:** Supervision, writing-review and editing. **A.U. Budzar:** Conceptualization, resources, writing-review and editing. **J.F.R. Robertson:** Conceptualization, resources, writing-review and editing. **W. Gradishar:** Conceptualization, resources, writing-review and editing. **M. Piccart:** Conceptualization, resources, writing-review and editing. **G. Schiavon:** Conceptualization, resources, writing-review and editing. **J.M. Bliss:** Conceptualization, resources, writing-review and editing. **M. Dowsett:** Conceptualization, resources, writing-review and editing. **S.R.D. Johnston:** Conceptualization, resources, writing-review and editing. **S.K. Chia:** Conceptualization, resources, writing-review and editing.

Acknowledgments

We thank the patients who participated in the SoFEA and EFECT studies, along with investigators and site staff. SoFEA (NCT00253422) and EFECT (NCT00065325) were supported by AstraZeneca. AstraZeneca provided financial assistance for *ESR1* mutational analysis in EFECT. Other funding was provided by Le Cure, Cancer Research UK funding to the ICR Clinical Trials and Statistics Unit, and Breast Cancer Now.

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Received January 24, 2020; revised May 11, 2020; accepted June 11, 2020; published first June 16, 2020.

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