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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

Nicholas C. Turner[1,2], Claire Swift[2], Lucy Kilburn[3], Charlotte Fribbens[1,2], Matthew Beaney[1], Isaac Garcia-Murillas[1], Aman Budzar[4], John F R Robertson[5], William Gradishar[6], Martine Piccart[7], Gaia Schiavon[8], Judith M Bliss[3], Mitch Dowsett[1,2], Stephen Johnston[+2], and Stephen Chia[+9].

1. Breast Cancer Now Research Centre, The Institute of Cancer Research, London, UK; 2. Breast Unit, The Royal Marsden Hospital, London, UK; 3. ICR-CTSU, The Institute of Cancer Research, London, UK; 4. MD Anderson Cancer Center, Houston, Texas, USA; 5. University of Nottingham, Nottingham, UK; 6. Northwestern University, Chicago, USA; 7. Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; 8. R&D Oncology, AstraZeneca, Cambridge, UK; 9. British Columbia Cancer Agency, Vancouver, Canada

[+]Authors contributed equally to this manuscript

Corresponding author:
Nicholas Turner, The Institute of Cancer Research and Royal Marsden, Fulham Road, London, SW3 6JB, UK, Email: nicholas.turner@icr.ac.uk

Running title. *ESR1* mutations and overall survival

Conflict of interest statement

Nicholas Turner has received advisory board honoraria from Astra Zeneca, Bristol-Myers Squibb, Lilly, Merck Sharpe and Dohme, Novartis, Pfizer, Roche/Genentech, Tesaro, Bicycle Therapeutics and research funding from Astra Zeneca, BioRad, Pfizer, Roche/Genentech, Clovis, and Guardant Health. John Robertson has received consulting fees from AstraZeneca, Bayer, Novartis, Cullinan Therapeutics, Carrick Therapeutics and Oncimmune; and performed contracted research on behalf of AstraZeneca, Bayer, Novartis, and Oncimmune; has given expert testimony for AstraZeneca; and holds stock with Oncimmune and stock options in Carrick Therapeutics. Gaia Schiavon is an employee of AstraZeneca UK and shareholder. Mitch Dowsett has received advisory board honoraria from Radius, lecture fees from

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Abstract

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

Patients and Methods. The phase III EFECT and SoFEA trials randomised patients with HR+ metastatic breast cancer who had progressed on prior non-steroidal AI, between fulvestrant 250mg and exemestane. Baseline serum samples from 227 patients in EFECT, and baseline plasma from 161 patients in SoFEA, were analysed for *ESR1* mutations by digital PCR. The primary objectives were to assess the impact of *ESR1* mutation status on progression-free and overall survival 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%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,0.81-1.37; p=0.69). There was an interaction between *ESR1* mutation and treatment (p=0.02). Patients with *ESR1* mutation detected had one-year overall survival 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 has one-year overall survival 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 associated with inferior progression-free and overall survival in patients treated with exemestane versus fulvestrant.

Statement of translational relevance

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

Introduction

Mutations in the oestrogen 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 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 investigated the optimal second line endocrine therapy, randomising patients progressing on a non-steroidal aromatase inhibitor between fulvestrant 250mg and exemestane, the SoFEA[9] and EFECT[10] studies. In prior analysis we analysed *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% 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)[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].

To assess the clinical utility of *ESR1* mutation analysis in ctDNA, we analysed 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.

Methods

Study designs

The EFECT study was a randomised placebo controlled double-dummy phase III trial conducted in post-menopausal women with advanced hormone receptor–positive breast cancer who had previously progressed on a non-steroidal aromatase inhibitor. Patients were randomized between fulvestrant 250mg 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%, Figure 1). The subset of patients with baseline serum available had similar baseline characteristics and outcome to patients without samples (Supplementary tables 1 and 2).

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 prior non-steroidal AIs, 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 analysed in 161 baseline plasma samples as previously described[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 previously described[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[3] (Supplementary figure 1). DNA was extracted from up to 1 ml of baseline samples and analysed 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 progression-free survival between *ESR1* mutation status and treatment randomisation between exemestane and fulvestrant, and to assess overall survival in patients with baseline *ESR1* mutations detection. The primary endpoint of EFECT was time to progression (TTP, defined as objective disease progression or death) and the primary endpoint of SoFEA was progression-free survival (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 was progression-free survival (defined as in the original trial) and overall survival, in the subset of patients with successful mutation analysis and with treatment 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 AI 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 overall survival analysis in the *ESR1* mutation detected group was shown to be non-proportional, a restricted mean survival analysis with a cut-off of 24 months was also used for OS analysis. Secondary analyses included the association between detection of *ESR1* mutations and clinical and pathological 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, College Station, TX).

Results

ESR1 mutation analysis in EFECT

To enable a combined analysis of EFECT and SoFEA, we first analysed *ESR1* mutation status in baseline serum samples from EFECT (Supplementary figure 2). *ESR1* mutations were successfully analysed in 98% (222/227) of patients with baseline serum samples, with *ESR1* mutations detected in 23.4% (52/222) samples. (Supplementary figure 3).

ESR1 mutations and progression-free survival in the combined analysis

The combined analysis of SoFEA and EFECT comprised a total of 383 patients with baseline sample *ESR1* ctDNA results (Figure 1 and Supplementary table 3), with 326 PFS events. *ESR1* mutations were detected in 30% (115/383) baseline samples overall, more frequently in patients with sensitivity to prior AI as defined by the original trials (p=0.02) and by setting and time on prior AI 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) (Figure 2). There was a statistically significant interaction between treatment randomisation and *ESR1* mutation status (interaction p=0.02).

In a multivariable 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 progression free survival improvement with fulvestrant compared to exemestane (Supplementary Figure 4 and 5). Patients with monoclonal *ESR1* mutations had median PFS on fulvestrant 3.6 months (95% CI,2.7-5.7, N=70) and with polyclonal *ESR1* mutations had median PFS on fulvestrant 6.6 months (95% CI,2.9-11.7, N=42) (Supplementary Figure 6).

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's exact 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's exact 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's exact 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's exact p=0.56).

ESR1 mutations and adverse short-term overall survival

We investigated the association between *ESR1* mutation status and overall survival 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 fulvestrant (HR=0.89, 95%CI,0.64-1.23, p=0.49) (Figure 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's proportional hazards assumption was violated for patients with *ESR1* mutations detected (proportionality assumption rho=0.22, chi2=3.86, p=0.049), suggesting non-proportional hazards. We therefore repeated the OS analysis utilising 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- -0.1; p=0.04), with no difference in the cohort with no detectable *ESR1* mutation (mean difference=-0.8 95%CI,-2.5-0.9; p=0.35). The estimated rates of overall survival at one-year in patients with *ESR1* mutation detected was 62% (45%-75%) on exemestane and 80% (68%-87%) on fulvestrant (one year landmark analysis HR=0.50 95% CI,0.24-1.04, p=0.06, Figure 3). In patients without *ESR1* mutations detected the one-year overall survival was 79% (71%-85%) on exemestane and 81% (74%-87%) on fulvestrant (p=0.75, Figure 3).

Discussion

We conducted a combined analysis of EFECT and SoFEA to investigate the clinical impact of *ESR1* mutation analysed in circulating tumor DNA. Patients with *ESR1*

mutations detected had shorter progression-free survival when treated with exemestane therapy, compared with fulvestrant, and also had shorter overall survival in restricted mean survival analysis. Analysis of overall survival in patients with *ESR1* mutations suggested non-proportional 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[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 progression-free survival (95%CI,7.5–14.3)[14], and tamoxifen plus everolimus showed substantial activity in the phase II TamRAD trial with 8.6 months progression-free survival (95%CI,5.9-13.9)[15]. In an exploratory analysis of the TamRAD study, there was an overall survival improvement with tamoxifen plus everolimus (HR=0.45, 95%CI,0.24-0.84, P=0.007)[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)[16]. Although TamRAD was a relatively small phase II study, we speculate that the overall survival 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 pre-clinical evidence that *ESR1* mutant cancer is resistant to oestrogen deprivation[17, 18], our data suggests 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 Figure 5). Although patients with polyclonal *ESR1* mutations in this data set had numerically improved outcome on fulvestrant compared to monoclonal *ESR1* mutations (Supplementary Figure 6), this analysis is limited by small numbers, and other data sets have not shown improved outcome of polyclonal versus monoclonal

on fulvestrant[19]. In the future further investigation of whether different *ESR1* mutations have differing responsiveness to fulvestrant, or tamoxifen, will be useful in this regard[18].

It is important to emphasise that patients treated with fulvestrant in EFECT and SoFEA were treated with a dose half that used currently, fulvestrant 250mg versus fulvestrant 500mg in the CONFIRM study[20], and this has important implications for interpreting the results of patients with undetected *ESR1* mutations. Although there was no difference between exemestane and fulvestrant (Figure 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 500mg compared with exemestane[20]. One other point from our results is that if post-AI a clinician is considering prescribing fulvestrant 500mg there is likely no utility in assessing *ESR1* mutations at that point. AI 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 AI 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 manuscript and BEAMing digital PCR[21], providing evidence of inter-assay agreement when conducted in central laboratories. Further research is required on the widespread clinical application of such assays. In EFECT we analysed serums 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 figure 1), 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 to 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 CDK4/6 inhibitor and AI also acquire *ESR1* mutations, possibly at approximately the same incidence to patients on AI alone, although an accurate incidence has not yet been established [22, 23]. 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 circulating tumor DNA analysis predicts lack of benefit from subsequent aromatase inhibitor therapy. Patients with *ESR1* mutations acquired through prior AI therapy have both adverse progression-free and overall survival 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, to aid in selection of the most appropriate subsequent endocrine therapy backbone, if further aromatase inhibitor-based therapy is being considered.

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

	ESR1 mutant *N*=115	ESR1 wildtype *N*=268
Age at randomisation (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	73 (63.5)	155 (57.8)
Yes	42 (36.5)	113 (42.2)
No		
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 randomisation (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 & 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)

ER – oestrogen receptor; PgR – progesterone receptor; ABC – advanced breast cancer; NSAI – non steroidal aromatase inhibitor; AI status – sensitivity to prior aromatase inhibitor (sensitive indicating relapse after at least 2 years adjuvant AI or response or duration of stable disease lasting at least 24 weeks in the metastatic setting). P values from Chi-squared test.

Table 2. Multivariable analysis of progression free survival in the SoFEA and EFECT combined analysis

		Hazard ratio (95% CI)	p value
***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 randomisation	<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 randomisation	<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 X Treatment group (interaction)**		0.61 (0.38, 1.00)	0.05

ER – oestrogen receptor; PgR – progesterone receptor; ABC – advanced breast cancer.

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

Figure 2. Progression free survival 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, Mutant – *ESR1* mutation detected, Wild type – *ESR1* mutation not detected.

Figure 3. Overall survival 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. For patients without *ESR1* mutation detected, restricted mean survival analysis p=0.69. E – exemestane, F – fulvestrant, Mutant – *ESR1* mutation detected, Wild type – *ESR1* not detected.

Figure 1

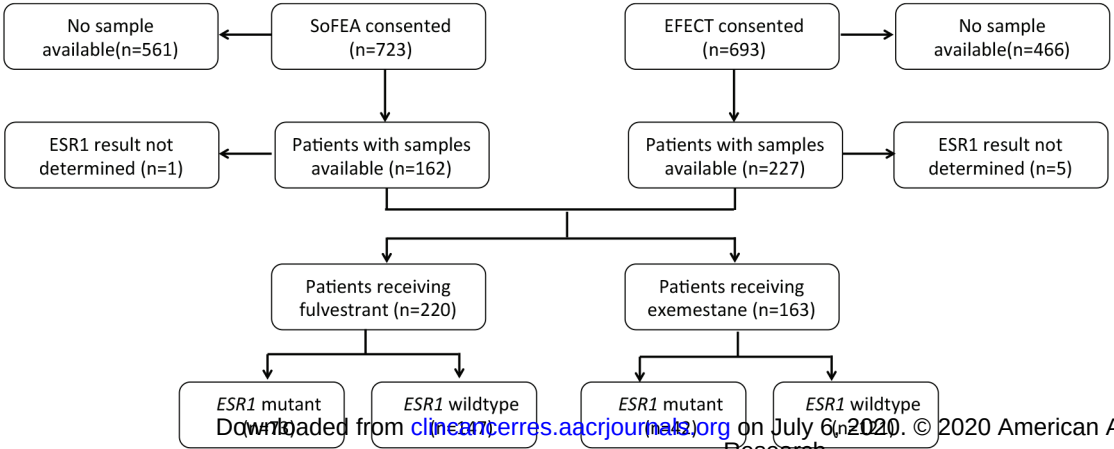
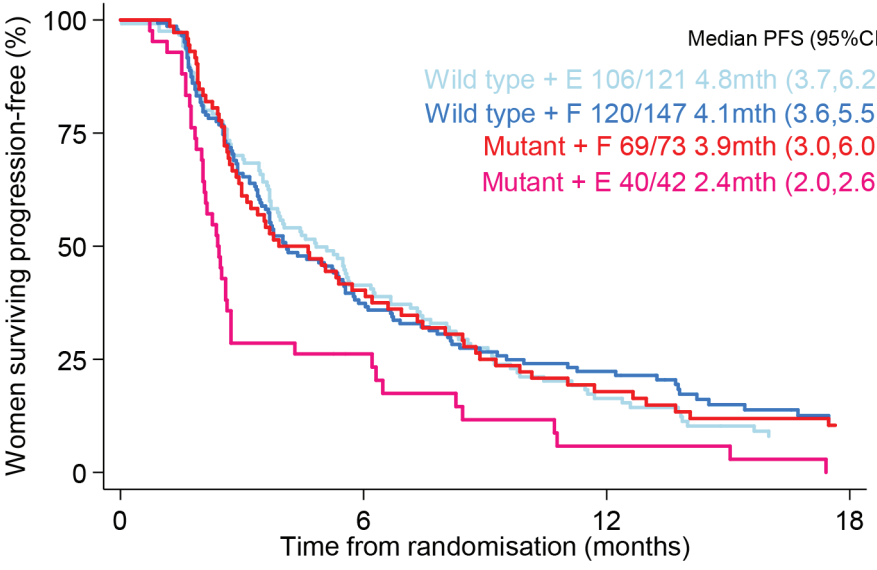


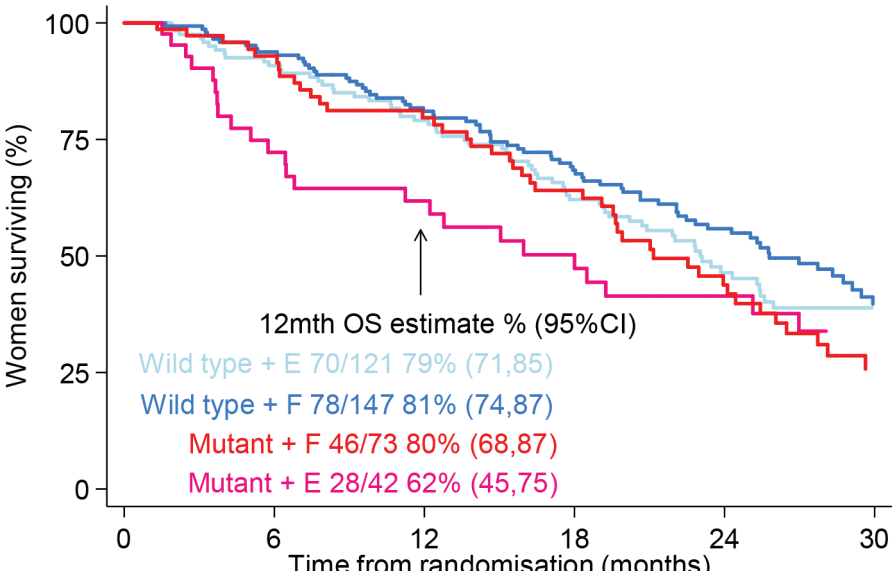
Figure 2



N at risk (events)

Wild type + E	121	(70)	49	(28)	17	(8)	7
Wild type + F	147	(87)	50	(19)	25	(10)	8
Mutant + E	42	(31)	9	(7)	2	(2)	0
Mutant + F	73	(43)	29	(16)	12	(5)	6

Figure 3



N at risk (events)

Wild type + E	121	(11)	109	(14)	93	(19)	68	(15)	37	(6)	21
Wild type + F	147	(9)	134	(18)	113	(17)	89	(15)	59	(13)	26
Mutant + E	42	(11)	28	(4)	22	(4)	17	(3)	11	(2)	6
Mutant + F	73	(5)	64	(9)	52	(10)	38	(11)	23	(8)	9

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Nicholas C. Turner, Claire Swift, Lucy S Kilburn, et al.

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