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ORIGINAL RESEARCH

Incidence and clinical significance of *ESR1* mutations in heavily pretreated metastatic breast cancer patients

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Background: *ESR1* mutation has recently emerged as one of the important mechanisms involved in endocrine resistance. The incidence and clinical implication of *ESR1* mutation has not been well evaluated in heavily pretreated breast cancer patients.

Methods: We conducted a retrospective review of advanced breast cancer patients with tumors who underwent next-generation sequencing genomic profiling using Foundation One test at Cancer Treatment Centers of America® regional hospitals between November 2012 and November 2014.

Results: We identified a total of 341 patients including 217 (59%) estrogen receptor (ER)+, 177 (48%) progesterone receptor (PR)+, 30 (8%) hormone receptor+/HER2 positive, and 119 (32%) triple negative patients. *ESR1* mutation was noted in 27/222 (12.1%) ER+ or PR+ breast cancer patients. All ER+ patients received at least one line of an aromatase inhibitor. All 28 patients were found to harbor *ESR1* mutations affecting ligand-binding domain with the most common mutations affecting Y537 (17/28, 60.7%) and D538 (9/28, 32.1%). In this cohort, 19 (67.9%) patients carried three or more, seven (25%) patients had one or two additional genomic alterations and one (3.6%) patient had an *ESR1* mutation only. Of 28 patients, three patients were treated with fulvestrant immediately before and two patients were treated after next-generation sequencing testing; only one patient achieved stable disease for 8 months and the other four patients had progression of disease. In all, 3/3 (100%) patients before testing and 2/4 (50%) after testing treated with exemestane and everolimus achieved stable disease for at least 6 months.

Conclusion: *ESR1* mutation was found in 12.1% of a large cohort of advanced breast cancer patients. Exemestane in combination with everolimus might be a reasonable option. Prospective studies are warranted to validate these findings.

Keywords: *ESR1* mutation, breast cancer, endocrine therapy, resistance, next-generation sequencing, genomic alteration

Introduction

Breast cancer is the leading cause of cancer deaths in women worldwide. It is projected that over 230,000 cases of invasive breast cancer will be diagnosed in the US and more than 40,000 patients are expected to die of metastatic disease in 2015.[1] Five-year survival of patients with stage IV metastatic breast cancer is approximately 25%.[1] Among those with advanced breast cancer, 60%–70% of the patients have estrogen receptor (ER)+ disease. ERα is a nuclear transcription factor that drives proliferation and growth of ER+ breast cancers. Endocrine therapy has been widely accepted as the cornerstone treatment due to its antitumor activity and favorable side effect profile. Unlike tamoxifen working through

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ER blockade, aromatase inhibitors (AIs) are primary directed at reducing estrogen synthesis and typically used as first-line hormonal therapy particularly in postmenopausal women with metastatic disease.[2,3] Unfortunately, approximately 30%–40% patients do not respond to the therapy due to intrinsic or de novo resistance.[2,3] Even for the initial responders, the objective response rate was only 20%–30%, and the median disease-free survival was quite short, ranging from 6 to 11 months likely as a result of acquired resistance.[2,4,5]

The development of endocrine resistance (de novo or acquired) poses a significant clinical challenge. After progression on AIs, second-line hormonal therapy with a steroidal AI such as exemestane or an ER downregulator fulvestrant benefited only 30% of patients with a response rate of merely 7% as demonstrated in the EFECT trial.[5] This result was further confirmed by the CONFIRM trial, albeit a higher dose of fulvestrant conferred a slightly higher response rate and overall survival advantage.[6,7] The underlying mechanisms for endocrine resistance remain to be elucidated. Putative mechanisms of acquired resistance include estrogen-independent growth, hypersensitivity to low estrogen concentrations, upregulation of the phosphatidylinositol 3-kinase–AKT–mammalian target of rapamycin pathway, *cyclin D1* overexpression, downregulation of ERα expression, and so on.[8,9] These pathways provide potential targets for therapeutic intervention to restore sensitivity to endocrine resistance. Everolimus, a mammalian target of rapamycin inhibitor, was demonstrated to overcome endocrine resistance in some patients when used in combination with exemestane in the BOLERO-2 study.[10] Exemestane combined with everolimus has been widely adopted as one of the second-line options by clinical guidelines. Palbociclib, a cyclin-dependent kinase 4/6 inhibitor, is another agent which has shown activity in ER+ breast cancer.[11]

Recently, *ESR1* mutation has started to emerge as another potential mechanism implicated in acquired endocrine resistance. The mutations in ligand-binding domain (LBD) create a ligand-free constitutively activated ER.[12] The reported incidence of the mutation was as low as less than 1% in primary tumor and as high as 11%–55% in metastatic ER+ breast cancer.[10,13–15] To confirm this finding and explore the role of further endocrine therapy in a large cohort of patients with heavily pretreated advanced breast cancer, we conducted a retrospective review of all patients with advanced breast cancer whose tumors underwent next-generation sequencing (NGS) genomic profiling using the Foundation One (FO) test.

Methods

Study population

This was a retrospective analysis of tumors from 341 advanced breast cancer patients who received treatment at one of the five Cancer Treatment Centers of America® regional hospitals. The inclusion criterion was a recurrent breast cancer which had failed to respond to or progressed on at least two lines of standard therapy and underwent a biopsy in either locoregional or metastatic sites. All samples were stained by immunohistochemistry for ER, progesterone receptor (PR), and human epidermal growth receptor 2 (HER2) and reviewed by a pathologist at each institution. ER and PR positivity was defined as more than 1% of cells with strong staining. HER2 positivity was defined as either by immunohistochemistry of 3+ or Fluorescent In Situ Hybridization HER2/CEP17 ratio of greater than 2.2. The paraffin-embedded blocks from biopsy specimens between November 2012 and November 2014 were then sent for NGS genomic profiling using the FO test. The captured clinical information included: age, hormone receptor status, histology, site of origin of the tumor sample and hormonal and/or chemotherapy received, and outcome. This retrospective analysis was approved by the Institutional Review Board at the Cancer Treatment Centers of America. This study involved the use of data and records that already existed, and did not involve taking additional biological samples from the patients, therefore patient consent was not required.

NGS genomic profiling

Targeted NGS was performed by Foundation Medicine in a Clinical Laboratory Improvement Amendment-certified laboratory. The targeted NGS platform FO has been previously described and validated.[16] FO testing utilizes NGS to identify alterations in genes known to be somatically altered in human solid cancers. FO is a mid/large gene panel which currently interrogates up to 315 cancer-related genes and 28 genes commonly rearranged in cancer. It detects all classes of genomic alterations, including base substitutions, insertions and deletions, and copy number alterations and rearrangements using a small, routine tumor sample. *ESR1* mutation is one of the genomic alterations reported on each analysis.

Statistical analysis

Descriptive statistics (means and frequencies) for baseline clinical and pathological characteristics were calculated.

Results

Incidence of *ESR1* mutation

ESR1 mutations were identified in 28 patients with breast cancer. Table 1 describes the characteristics of the breast cancer

Table 1 Characteristics of breast cancer patients with *ESR1* mutations (n=28)

	Number of patients with *ESR1* mutation	Total number of patients tested	% of patients
ER+	27	217	12.5
PR+	24	177	13.6
ER+ or PR+	27	222	12.1
ER+/HER2	2	30	6.7
Triple negative	1*	119	0.8
Specimen site			
Primary breast tumor	0		0.0
Local recurrence	3		10.7
Metastasis	25		89.3

Note: *This patient with ER+ primary breast cancer presented with lung metastases and biopsy of one of the lung metastases revealed a triple negative breast cancer.
Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth receptor 2; PR, progesterone receptor.

patient cohort. In all, 27 patients harboring *ESR1* mutations were identified in 217 ER+ patients (27/217=12.45%); 24 of these 27 patients were also PR+, identified in 177 PR+ patients (24/177=13.6%). Taken together, the incidence of *ESR1* mutation was 12.1% (27/222) in HR+ (ER+ or PR+) patients. The incidence of *ESR1* mutations in both ER+ and HER2 positive patients was 6.7% (2/30). Only one of 119 triple negative breast cancer (TNBC) samples was found to carry an *ESR1* mutation (1/119=0.8%) and the primary tumor of this patient was ER+. Therefore, based on our results it appears that the incidence of *ESR1* mutation in TNBC was very small. Most of the breast cancer patients were heavily pretreated, having received on average at least two lines of cytotoxic chemotherapy in the metastatic setting and three lines of hormonal therapy including adjuvant hormonal therapy as shown in Table 1. All patients received at least one line of an AI. Median age of the 28-patient cohort was 55.5, ranging from 34 to 75. The specimen sources for FO analysis included local recurrence (n=3, 10.7%) and distant metastases (n=25, 89.3%).

ESR1 mutations and other genomic alterations in metastatic breast cancer

All 28 patients were found to harbor *ESR1* mutations affecting LBD, with the most common mutations affecting Y537 (17/28, 60.7%) and D538 (9/28, 32.1%). In all, 11 (11/28, 39.3%) patients had an *ESR1* Y537S mutation, four (4/28, 14.3%) an *ESR1* Y537C mutation, and two (2/28, 7.1%) an *ESR1* Y537N mutation. Eight (8/28, 28.6%) harbored an *ESR1* D538G mutation, and one (1/28, 3.6%) patient had an *ESR1* D538G mutation as well as amplification. One patient had mutations involving three amino acids from 536 to 538 (L536PY537PD538P), which has never been reported before. One patient carried an *ESR1* V533M mutation (Table 2). We also examined the frequency of additional genomic alterations other than *ESR1* mutations in this heavily pretreated cohort of patients. We found that 19 (67.9%) patients carried three or more genomic alterations, seven (25%) patients had one or two additional genomic alterations, and two (7%) patients had an *ESR1* mutation only (Table 2). The most common genomic alteration involved *PIK3CA*, *GATA3*, *cyclin D1*, *fibroblast growth factors*, and *fibroblast growth factor receptor 1* (Table 3) genes.

Clinical outcome after treatment with endocrine therapy

Of 28 patients, 15 patients received at least one line of chemotherapy either immediately before or after FO testing and therefore were excluded from the assessment of the endocrine therapy outcome. As a result, only 13 patients were eligible

Table 2 Summary of *ESR1* mutation and other genomic alterations (GAs) (N=28)

ESR1 and GA	Patients, n	% patients affected	*ESR1* only	1 additional GA	2 additional GA	>2 additional GA
ESR1 Y537S	11	39.3	0	1	2	8
ESR1 D538G	8	28.6	1	2	1	4
ESR1 Y537C	4	14.3	0	1	0	3
ESR1 Y537N	2	7.1	0	0	0	2
ESR1 amplification, D538G	1	3.6	0	0	0	1
ESR1 L536_D538>P	1	3.6	0	0	0	1
ESR1 V533M	1	3.6	1	0	0	0
Total	28	100.0	2	4	3	19

Table 3 Distribution of additional genomic alterations (GAs) with frequency ≥2

Additional GA	Frequency	% of patients, n=28	Additional GA	Frequency	% of patients, n=28
PIK3CA	10	35.71	*TET2*	3	10.71
GATA3	6	21.43	*ZNF703*	3	10.71
CCND1	5	17.86	*ARID1A*	2	7.14
FGF3	5	17.86	*BRCA2*	2	7.14
FGF4	5	17.86	*FANCA*	2	7.14
FGF19	5	17.86	*TP53*	2	7.14
FGFR1	5	17.86	*ZNF217*	2	7.14
MYST3	4	14.29	*CDH1*	2	7.14
IKBKE	3	10.71	*MDM4*	2	7.14
PTEN	3	10.71	*MYC*	2	7.14

Note: Genes are listed by frequency.

for this exploratory analysis. Three patients were treated with fulvestrant immediately before and two patients were treated after NGS testing. Only one patient achieved stable disease for 8 months and the other four patients had progression of disease, demonstrating a clinical benefit rate of 20% (1/5). One patient treated with tamoxifen right before FO testing did not respond. However, 3/3 (100%) patients before testing and 2/4 (50%) after testing treated with exemestane and everolimus achieved stable disease for at least 6 months (Table 4).

Discussion

In this study, we identified 28 patients with *ESR1* mutations from 341 patients with advanced breast cancer. To our knowledge, this represents the largest reported study of endocrine-refractory breast cancer patients with *ESR1* mutations. The first case of *ESR1* mutation was described in 1997 in a patient with metastatic breast cancer.[17] Subsequently, the Cancer Genome Atlas Research Network failed to identify any *ESR1* mutations in primary breast cancer.[18] Recently, two independent studies reported somatic *ESR1* mutations in six of eleven (55%) and nine of 36 (25%) patients with ER+ metastatic breast cancer, respectively.[13,14] Jeselsohn et al studied ER+ samples consisting of 58 primary and 76 metastatic breast cancers. No *ESR1* mutations were detected in primary ER+ cancers, but nine of 76 (12%) of metastatic samples were found to carry *ESR1* mutations.[19] These findings suggest that *ESR1* mutations in treatment-naïve patients are a rare event; however, it is relatively common to harbor *ESR1* mutations in ER+ metastatic breast cancer patients. The incidence of *ESR1* mutation has not been well established, ranging from 12% to 55%.[14,15,19] Toy et al reported that five of 44 (11%) ER+ breast cancer patients with disease progression during treatment with AIs carried *ESR1* mutations in the BOLERO-2 clinical trial.[14] The incidence of *ESR1* mutation in this study appears to be consistent with the 12% (9/76) reported by Jeselsohn et al[19] where all but two patients have previously received AIs. In our study, we identified 12.1% (27/222) of patients with an *ESR1* mutation in heavily pretreated, HR+ breast cancer patients (Table 1). Taken together, these three studies suggest that the incidence of *ESR1* mutations in the pretreated

Table 4 *ESR1* mutation and endocrine therapy immediately before or after testing (n=13)

Patients and GAs	Pretest endocrine therapy	Response
#2 D538G +2 GAs	Exemestane + everolimus	SD for 6 months
#3 L536_D538>P +5 GAs	Exemestane + everolimus	SD for 8 months
#4 Y537C +8 GAs	Fulvestrant	SD for 8 months
#5 D538G +3 GAs	Exemestane + everolimus	SD for 12 months
#8 Y537N +7 GAs	Fulvestrant	PD
#10 Y537S +1 GA	Tamoxifen	PD
#12 Y537S +4 GA	Fulvestrant	PD
Patients and GAs	**Post-test endocrine therapy**	**Response**
#17 Y537S +8 GAs	Exemestane + everolimus	PD
#21 Y537S +3 GAs	Exemestane + everolimus	PD
#22 D538G +4 GAs	Exemestane + everolimus	SD for 18 months
#23 D538G +4 GAs	Exemestane + everolimus	SD for 6 months
#30 Y537S +2 GAs	Fulvestrant	PD
#31 D538G +0 GA	Fulvestrant	PD

Abbreviations: GA, genomic alteration; PD, progression of disease; SD, stable disease.

ER+ metastatic breast cancer patient is approximately 12%. Thus, *ESR1* mutation appears to be a frequently acquired mutation in the metastatic setting and in particular in patients who have previously received AIs. Given the prevalence of ER+ breast cancer, advanced breast cancer harboring *ESR1* mutations would be expected to affect a large population of patients and therefore warrants further studies.

All 28 patients were found to harbor *ESR1* mutations affecting LBD, with the most common mutations affecting Y537 (17/28, 60.7%) and D538 (9/28, 32.1%) as shown in Tables 2 and 3. Preclinical studies have previously shown that LBD mutations result in constitutive, ligand-independent ER activity, involved in endocrine resistance.[13,14,19] All 28 patients identified in our study carrying *ESR1* mutation failed at least one line of endocrine therapy with an AI, suggesting *ESR1* mutation is at least partially responsible for endocrine resistance. The clinical implications to overcome the resistance conferred by *ESR1* mutations would be significant. In vivo experiments in breast cancer cell lines demonstrate that high-dose tamoxifen and fulvestrant were shown to inhibit the activity of *ESR1* with mutations in LBD.[14,19] However, there are no prospective studies available that specifically address this issue at this time. In the CONFIRM trial, higher doses of fulvestrant increased progression-free survival and overall survival after patients failed prior endocrine therapy.[6,7] The underlying mechanisms are still poorly understood. It is conceivable that some patients may have acquired *ESR1* mutations and, as a result, a higher dose of fulvestrant worked more effectively and contributed to longer progression-free survival and overall survival. In our cohort of patients, in five patients treated with a standard dose of fulvestrant (500 mg every 4 weeks after loading dose) immediately before or after NGS testing, only one patient achieved stable disease for 8 months. One patient treated with tamoxifen right before NGS testing did not respond (Table 4). Ideally, it is preferable to assess all patients who received endocrine therapy immediately after NGS testing, but in clinical practice it is extremely challenging to identify enough patients. However, if a patient progressing on endocrine therapy was tested positive for ESR mutation, it is self-evident that this patient will not respond to the very same therapy after the testing. The converse could also be true if a patient were responding clinically on endocrine therapy and tested positive for ESR mutation. Thus, we included the patients who received endocrine therapy immediately before NGS for this exploratory analysis. In our study, although the number of patients is small, the clinical benefit rate is certainly lower than what was reported in the CONFIRM trial.[6,7] It also seems to suggest that it is not very promising to treat *ESR1* mutation carriers with a standard dose of fulvestrant or tamoxifen. In contrast, 3/3 (100%) patients before testing and 2/4 (50%) after testing treated with exemestane and everolimus achieved stable disease for at least 6 months (Table 4). The clinical benefit rate of 70% (5/7) seems to be well aligned with what was reported by the BOLERO-2 study where the clinical benefit rate was approximately 51%. It would be interesting to find out how the subpopulation of patients who tested positive for *ESR1* mutations responded to exemestane and everolimus in this study. The etiology for the different response to fulvestrant versus exemestane plus everolimus is unclear at this point. One could speculate that a standard dose of tamoxifen or fulvestrant is simply too low to be effective for *ESR1* mutation patients. On the other hand, *ESR1* mutation alone appeared to be a rare phenomenon in this heavily pretreated patient cohort, accounting for 7% (2/28) of the patients with *ESR1* mutations; 93% of the patients (26/28) had at least one additional genomic alteration other than *ESR1* mutations (Tables 2 and 3). Very likely, these genomic alterations represent alternative mechanisms of endocrine resistance; therefore, targeting upstream ER alone with fulvestrant or tamoxifen is simply not sufficient to suppress endocrine resistance likely derived from both constitutively active ER and/or downstream signaling molecules. Similar to what was demonstrated in the BOLERO-2 study, *PIK3CA*, *cyclin D1*, and *fibroblast growth receptor 1* were also the three most commonly altered genes in our study (Table 3).[20] Not surprisingly, the combination of everolimus, plus exemestane was able to achieve stable disease in five of seven patients without targeting specifically against *ESR1* mutations. As such, in order to effectively target *ESR1* mutations, it may require incorporating both effective ER targeting such as a higher dose of fulvestrant or a more potent analog and targeted therapy of downstream components. Most recently, the PALOMA-3 study demonstrated that the combination of palbociclib with fulvestrant improved progression-free survival in HR+ advanced breast cancer patients who had progressed on prior endocrine adjuvant therapy. Biomarker analysis including *ESR1* mutations is eagerly awaited.[21]

Conclusion

In summary, our study demonstrates an overall 12% frequency of *ESR1* mutations in the largest cohort of advanced breast cancer patients through NGS of metastatic lesions. Moreover, the majority of the patients harbored at least one

additional genomic alteration. Prospective studies are needed to better understand and overcome *ESR1*-derived endocrine resistance in advanced ER+ breast cancer patients using more effective anti-ER agents in combination with targeted therapy toward downstream components.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015;65(1):5–29.
2. Nabholtz JM, Buzdar A, Pollak M, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. *J Clin Oncol*. 2000;18(22):3758–3767.
3. Mouridsen H, Gershanovich M, Sun Y, et al. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. *J Clin Oncol*. 2003; 21(11):2101–2109.
4. Giatromanolaki A, Koukourakis MI, Simopoulos C, et al. c-erbB-2 related aggressiveness in breast cancer is hypoxia inducible factor-1alpha dependent. *Clin Cancer Res*. 2004;10(23):7972–7977.
5. Chia S, Gradishar W, Mauriac L, et al. Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT. *J Clin Oncol*. 2008;26(10):1664–1670.
6. Di Leo A, Jerusalem G, Petruzelka L, et al. Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. *J Clin Oncol*. 2010;28(30):4594–4600.
7. Di Leo A, Jerusalem G, Petruzelka L, et al. Final overall survival: fulvestrant 500 mg vs 250 mg in the randomized CONFIRM trial. *J Natl Cancer Inst*. 2014;106(1):djt337.
8. Arpino G, De Angelis C, Giuliano M, et al. Molecular mechanism and clinical implications of endocrine therapy resistance in breast cancer. *Oncology*. 2009;77 Suppl 1:23–37.
9. Yardley DA, Ismail-Khan RR, Melichar B, et al. Randomized phase II, double-blind, placebo-controlled study of exemestane with or without entinostat in postmenopausal women with locally recurrent or metastatic estrogen receptor-positive breast cancer progressing on treatment with a nonsteroidal aromatase inhibitor. *J Clin Oncol*. 2013;31(17):2128–2135.
10. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. 2012;366(6):520–529.
11. Finn RS, Crown JP, Lang I, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol*. 2015;16(1):25–35.
12. Jordan VC, Curpan R, Maximov PY. Estrogen receptor mutations found in breast cancer metastases integrated with the molecular pharmacology of selective ER modulators. *J Natl Cancer Inst*. 2015;107(6):djv075.
13. Robinson DR, Wu YM, Vats P, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet*. 2013;45(12): 1446–1451.
14. Toy W, Shen Y, Won H, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet*. 2013;45(12):1439–1445.
15. Roodi N, Bailey LR, Kao WY, et al. Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. *J Natl Cancer Inst*. 1995;87(6):446–451.
16. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023–1031.
17. Zhang QX, Borg A, Wolf DM, et al. An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer Res*. 1997;57(7):1244–1249.
18. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61–70.
19. Jeselsohn R, Yelensky R, Buchwalter G, et al. Emergence of constitutively active estrogen receptor-alpha mutations in pretreated advanced estrogen receptor-positive breast cancer. *Clin Cancer Res*. 2014;20(7):1757–1767.
20. Hortobagyi GN. Everolimus plus exemestane for the treatment of advanced breast cancer: a review of subanalyses from BOLERO-2. *Neoplasia*. 2015;17(3):279–288.
21. Turner NC, Ro J, Andre F, et al. PALOMA3: a double-blind, phase III trial of fulvestrant with or without palbociclib in pre- and postmenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer that progressed on prior endocrine therapy. *J Clin Oncol*. 2015;33 abstr LBA502.