Strategies to overcome endocrine therapy resistance in hormone receptor-positive advanced breast cancer

Use of endocrine therapies has made hormone receptor-positive breast cancer a manageable disease if diagnosed at an early stage. However, endocrine therapy resistance is a persistent problem in patients with advanced breast cancer. Ongoing research has identified a number of mechanisms that may mediate resistance, including estrogen-independent activation of the estrogen receptor; increased signaling through the RAS/MAPK, NF-κB, or PI3K/Akt/mTOR pathway; and maintenance of cyclin D1 expression. Based on such findings, various strategies to overcome endocrine resistance have been developed. Although some therapies are in early development, others are available in the clinic. The novel treatment strategies under evaluation in clinical trials for managing patients with endocrine-resistant advanced breast cancer will be reviewed in combination with endocrine therapy.

Keywords: endocrine resistance • growth factor receptor • hormone receptor-positive • PI3K pathway • RAS/MAPK pathway • tyrosine kinases

In 2013, approximately 232,000 women will be diagnosed with invasive breast cancer (BC) in the USA and 40,000 will die of the disease [1]. Approximately 70% of BCs are hormone (estrogen and/or progesterone) receptor-positive (HR+) [2], and patients with HR+ BC have a better prognosis, in part, because of their responsiveness to endocrine therapies [3,4]. Tamoxifen, a selective estrogen receptor (ER) modulator, is a partial ER antagonist, initially used as first-line therapy in patients with HR+ advanced BC (ABC) [201]. Aromatase inhibitors (AIs) lower endogenous estrogen levels by inhibiting androgen to estrogen conversion [5]. The three third-generation AIs – anastrozole, letrozole and exemestane – were shown to be superior to tamoxifen in terms of efficacy and safety in postmenopausal women with ABC [6–12]. In addition, approximately 40% of patients treated with adjuvant endocrine therapy and almost all patients with metastatic BC (MBC) die of the disease [13].

Overview of endocrine resistance & strategies to overcome resistance

Endocrine resistance is a significant problem in treating BC. Patients can present with primary/acquired resistance (no response to initial endocrine therapy), or disease progression or recurrence can develop while the patient is receiving therapy (secondary/acquired resistance). In fact, approximately 30% of patients with MBC regress with initial endocrine therapy, whereas another 20% have prolonged stable disease [14]; and the duration of response to second and subsequent lines of therapy is substantially lower [15,16]. Since current endocrine therapy is effective, it is important to identify those patients who respond and those who do not respond to therapy in order to improve treatment decisions. However, predicting response can be challenging. Molecular profiling of snap-frozen tumor
biopsies has demonstrated that patients who recur early while on adjuvant tamoxifen therapy have a different molecular profile than those patients who experience a later recurrence while receiving tamoxifen [17]. In addition, molecular profiling analyses of patients who become resistant to aromatase inhibitors have found that patients who develop resistance to endocrine therapy have different gene expression patterns, suggesting that endocrine resistance is not a homogeneous phenomenon [18]. In addition, preclinical models have suggested that endocrine resistance results in upregulation of alternate signaling pathways that might be suitable targets for targeted therapies [19]. These data suggest that endocrine resistance could be a heterogeneous phenomenon that might necessitate molecular profiling to determine the appropriate course of action when resistance occurs. Additionally, because resistance might result in the upregulation of multiple pathways, the use of more than one therapy to target these pathways might be necessary.

Ongoing research has provided insight into the causes of endocrine resistance and a number of mechanisms have been proposed, such as loss of ERα (one of the two types of ER) expression through methylation; alterations in the expression of ER coactivators; and mutations in ERα [13,20]. However, no large-scale clinical data are available for most of these endocrine resistance mechanisms and they will not be discussed further.

Some of the mechanisms of endocrine resistance that have been studied in clinical trials (Figure 1) [21] include ligand (estrogen)-independent activation of ER, increased signaling through the RAS/MAPK pathway, NF-kB pathway, or PI3K/Akt/mTOR pathway, increased growth factor (GF) expression and signaling, and loss of ERα-mediated signaling through chromatin remodeling [13,20].

**Targeting the ER**

Unlike AIs and tamoxifen, fulvestrant, a selective ER down regulator, induces rapid degradation of ER [22]. In a Phase III trial in patients with previously untreated ABC comparing tamoxifen with fulvestrant at the initially evaluated dose of 250 mg every 28 days, now considered low-dose, fulvestrant was as effective, but not superior to, tamoxifen [23]. Although steady-state drug concentrations are reached only after 3–6 months with low-dose fulvestrant therapy, steady state levels were reached in less time using a loading dose consisting of 500 mg on day 1, 250 mg on days 14 and 28, and 250 mg every 28 days thereafter, and a high-dose, consisting of 500 mg on days 1, 14, and 28 of the first month and 500 mg every 28 days thereafter [24]. A Phase II, open-label trial (FIRST) in patients with ABC who may have had prior adjuvant endocrine therapy for early disease more than 12 months before randomization but with no previous endocrine therapy exposure for advanced disease, evaluated the efficacy of high-dose fulvestrant versus anastrozole [25]. Although there was no difference in the primary end point of clinical benefit rate (CBR) in the FIRST trial, the median time to progression (TTP) was substantially greater with high-dose fulvestrant (23.4 vs 13.1 months; hazard ratio: 0.66; 95% CI: 0.47–0.92; p = 0.01) [25,26]. An ongoing Phase III randomized trial is attempting to provide definitive evaluation of those results.

As AIs lower estrogen levels, endocrine resistance may result from development of estrogen-independent ER-mediated signaling [5]. Since fulvestrant promotes degradation of ERα through the ubiquitin–proteasome pathway in a preclinical setting [22], it was hypothesized that fulvestrant could overcome endocrine resistance. A combined analysis of two Phase III trials [27,28] in the second-line setting in postmenopausal women with locally ABC or MBC and disease progression during previous endocrine therapies, found low-dose fulvestrant as effective as anastrozole [29], leading to US FDA approval for use in postmenopausal women with HR+ ABC after progression on previous anti-estrogen therapy. In another Phase III trial, low-dose fulvestrant and exemestane were equally effective in patients with HR+ ABC after previous nonsteroidal AI therapy [30]. Recently, the effectiveness of high- and low-dose fulvestrant in postmenopausal women with ABC or MBC progressing during previous endocrine therapy has been directly compared in the Phase III (CONFIRM) trial. In that setting, high-dose fulvestrant, compared with low-dose, significantly improved median progression-free survival (PFS; 6.5 vs 5.5 months; hazard ratio = 0.80; 95% CI: 0.68–0.94; p = 0.006) [31] and overall survival (OS; 26.4 vs 22.3 months; hazard ratio = 0.81; 95% CI: 0.69–0.96; p = 0.016) [32]. Consequently, the FDA approved the high-dose fulvestrant schedule as a second-line therapy for postmenopausal women with HR+ metastatic disease [33].

Although preclinical models suggested that combining AI and fulvestrant may be an effective treatment option [34,35], clinical trial results have been conflicting. Results from the SWOG S0226 trial in postmenopausal women with previously untreated MBC, showed that low-dose fulvestrant plus anastrozole significantly improved median PFS (15.0 vs 13.5 months; p = 0.007) and median OS (47.7 vs 41.3 months; p = 0.049) than anastrozole alone [36]. In contrast, results from another Phase III trial (FACT) in postmenopausal women with HR+ ABC in the same setting, comparing low-dose
Figure 1. Mechanisms that may mediate endocrine resistance. (A) Ligand-bound ER activates gene expression either directly or through protein–protein interactions. (B) RTKs such as the EGFR, ERBB2 (also known as HER2) and the IGFR, can activate downstream signaling events, thereby regulating translation and transcription. (C) ER localized at the cell membrane or in the cytoplasm can mediate nongenomic signaling. (D) Formation of an ER–PI3K–Src–FAK complex can activate Akt, resulting in activation of downstream signaling cascade. (E) Activation of Erk by ER–Src–PELP1 complexes can activate downstream signaling events. All together these complex signaling cascades regulate growth, proliferation and survival, resulting in endocrine therapy resistance.

CoA: Coactivator; EGFR: EGF receptor; ER: Estrogen receptor; ERE: Estrogen response element; IGFR: IGF receptor; RE: Response element; RTK: Receptor tyrosine kinase; SRE: Serum response element; TF: Transcription factor.

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fulvestrant plus anastrozole with anastrozole alone, did not find statistically significant differences in TTP, CBR, objective response rate (ORR) or OS between the two treatments [37]. Results from another Phase III trial (SoFEA), comparing low-dose fulvestrant plus anastrozole with anastrozole alone, also found no differences in PFS, ORR, CBR or OS [38]. Therefore, current data are inconclusive for combining AI with low-dose fulvestrant to overcome endocrine resistance.

Inhibition of intracellular signaling cascade

In preclinical models, activation of intracellular signaling pathways (Figure 1) [21] — such as the PI3K/Akt/mTOR pathway, RAS/MAPK pathway, Src kinase signaling cascade, or NF-κB pathway — has been shown to mediate endocrine resistance. Strategies inhibiting one or more of these pathways in combination with endocrine therapies are under clinical evaluation.

Inhibition of the PI3K/Akt/mTOR pathway

Preclinical models of BC cells resistant to estrogen deprivation were found to have amplified PI3K/Akt/mTOR-mediated signaling [39], suggesting that activation of the PI3K pathway may facilitate survival of these cells under hormone deprivation. Additionally, by using cell lysates from hormone receptor-positive primary breast tumors, PI3K pathway activation was associated with poor disease outcome after adjuvant therapy [39]. Activation of PI3K results in activation of its downstream target Akt and in cells expressing activated Akt, the efficacy of tamoxifen to induce growth inhibition was dramatically reduced [40]. Since treatment with mTOR inhibitors reversed tamoxifen resistance in cells over expressing activated Akt [40,41] and reduced tumor growth in xenograft models [40], mTOR inhibitors (sirolimus, temsirolimus and everolimus) have been tested in clinical trials to overcome endocrine resistance.

Sirolimus & temsirolimus

Sirolimus, in combination with tamoxifen, has recently been shown to significantly improve median TTP and response rates compared with tamoxifen alone in a Phase II trial in postmenopausal women with HR+ ABC in whom previous tamoxifen and/or AI therapy was ineffective [42]. Although temsirolimus was effective in combination with letrozole in patients with ABC with disease progression during or after tamoxifen therapy in a Phase II trial [43], results from HORIZON, a Phase III trial in postmenopausal women with AI-naïve, locally advanced or MBC, did not show benefit for the temsirolimus combination compared with letrozole alone in the first-line setting, with results perhaps limited by the substantial toxicity seen [44].

Everolimus

In a Phase II neoadjuvant trial in patients with operable ER+ BC, everolimus in combination with letrozole improved the clinical response rate compared with letrozole alone [45]. In another Phase II trial, everolimus plus tamoxifen was compared with tamoxifen alone in postmenopausal patients with HR+, HER2+ MBC with previous exposure to AIs, and the combination significantly improved CBR (61 vs 42%; p < 0.045), TTP (8.6 vs 4.5 months; p < 0.002), and OS (hazard ratio: 0.45; 95% CI: 0.24–0.81; exploratory p = 0.007) [46].

In the BOLERO-2 Phase III trial evaluating everolimus plus exemestane versus exemestane alone in postmenopausal women with HR+, HER2+ ABC after previous letrozole or anastrozole, median PFS at 7.1 months was significantly improved with the everolimus combination (6.9 vs 2.8 months by local assessment; hazard ratio 0.43; 95% CI: 0.35–0.54; p < 0.001) [47]. The Kaplan–Meier plot shows substantial visual separation of the PFS curves at the 6-week reimaging period. Analyses from the 12.5- and 18-month follow-up data from BOLERO-2 produced similar results [48,49]. Based on the BOLERO-2 trial, everolimus (10 mg/day) in combination with exemestane (25 mg/day) was FDA approved for managing postmenopausal women with HR+ ABC and is currently the only approved mTOR inhibitor for managing patients with ABC after failure with letrozole or anastrozole [50]. In additional analyses from BOLERO-2, the everolimus combination was associated with a longer time to definitive deterioration of health-related quality of life (8.3 vs 5.8 months; hazard ratio: 0.74; p = 0.0084) [51] and was effective regardless of whether patients had visceral metastases [52]. Finally, exploratory analyses in the study found favorable effects on bone turnover, and less progression in bone metastases was seen for the everolimus combination [53].

Of potential clinical relevance are findings from the Phase III BOLERO-3 trial, in which everolimus (5 mg/day) addition was compared with trastuzumab and weekly vinorelbine alone in patients with HER2+ ABC [54]. Everolimus addition improved median PFS (hazard ratio: 0.78; p = 0.0067), but subgroup analyses suggested an effect limited to women with ER- disease (hazard ratio: 0.65; 95% CI: 0.49–0.86). These findings raise the hypothesis that combination therapy targeting both ER and HER2 pathways may be needed to optimize outcome in ER-, HER2+ ABC.

Ongoing trials

A Phase III adjuvant trial evaluating 1-year therapy with everolimus in addition to adjuvant endocrine therapy in high-risk patients with HR+ HER2- invasive BC is ongoing (Table 1) [202]. Other ongoing BC studies evaluating everolimus are also outlined in Table 1.
# Table 1. Ongoing clinical trials using PI3K/Akt/mTOR inhibitors to overcome endocrine resistance in patients with advanced breast cancer.

<table>
<thead>
<tr>
<th>Clinical trial identifier</th>
<th>Intervention</th>
<th>Patient population</th>
<th>Phase</th>
<th>Expected enrollment (n)</th>
<th>Select primary and secondary outcomes</th>
<th>Ref.</th>
</tr>
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</table>
| NCT01231659               | Letrozole + everolimus | Postmenopausal women with locally advanced BC or MBC after recurrence or progression on tamoxifen, anastrozole or exemestane | II    | 70                      | Primary: ORR  
Secondary: PFS, OS, DCR, safety          | [214]|
| NCT01499160               | Letrozole + lapatinib + everolimus | Postmenopausal women with endocrine-resistant advanced BC | II    | 76                      | Primary: CBR  
Secondary: PFS                           | [215]|
| NCT01797120               | Fulvestrant + everolimus | Postmenopausal women with AI-resistant MBC | II    | 130                     | Primary: PFS  
Secondary: ORR, TTP, OS toxicity           | [216]|
| NCT01698918               | Everolimus + letrozole | Postmenopausal women with ER+ MBC as first-line therapy | II    | 200                     | Primary: PFS  
Secondary: ORR, OS, reduction in severity and duration of stomatitis, safety | [217]|
| NCT01437566               | Fulvestrant ± GDC-0941 fulvestrant ± GDC-0980 | AI-resistant patients with advanced BC or MBC | II    | 270                     | Primary: PFS, safety  
Secondary: ORR, DOR, PK parameters           | [218]|
| NCT01783444               | Everolimus  
Capecitabine  
Everolimus + exemestane | Postmenopausal women with locally advanced BC, recurrent BC or MBC after recurrence or progression on prior letrozole or anastrozole | II    | 300                     | Primary: PFS  
Secondary: OS, ORR, CBR, change in ECOG status, QoL, safety | [219]|
| NCT01626222               | Exemestane + everolimus | Postmenopausal women with ER+, locally advanced BC or MBC progressing on prior therapies | IIIb  | 300                     | Primary: ORR  
Secondary: PFS, OS, safety, HR-QoL, resource use | [220]|
| NCT01633060               | Fulvestrant ± BKM120 | Postmenopausal women with HR+ HER2+ AI treated with locally advanced BC or MBC progressing on or after mTOR inhibitor therapy | III   | 615                     | Primary: PFS  
Secondary: OS, ORR, CBR, safety, PK, QoL | [221]|
| NCT01610284               | Fulvestrant ± BKM120 | Postmenopausal women with HR+ HER2+ AI treated with locally advanced BC or MBC refractory to AI therapy | III   | 842                     | Primary: PFS  
Secondary: OS, ORR, CBR, safety, PK, QoL | [222]|
| NCT01674140               | Adjuvant hormone therapy ± everolimus | High-risk patients with HR+, HER2+ BC | III   | 3500                    | Primary: IDFS assessed up to 10 years  
Secondary: OS, DRFS assessed up to 10 years, toxicity | [202]|

AI: Aromatase inhibitor; BC: Breast cancer; CBR: Clinical-benefit rate; DCE: Disease-control rate; DOR: Duration of response; DRFS: Distant recurrence-free survival; ECOG: Eastern Cooperative Oncology Group; ER: Estrogen receptor; HR: Hormone receptor; HR-QoL: Health-related QoL; IDFS: Invasive disease-free survival; MBC: Metastatic breast cancer; ORR: Overall-response rate; OS: Overall survival; PFS: Progression-free survival; PK: Pharmacokinetic; QoL: Quality of life; TTP: Time to progression.
Identifying biomarkers of response to everolimus
A key aspect of administering everolimus therapy is determining which patients would benefit most from treatment. However, identification of appropriate biomarkers has been challenging. Although PI3K-Akt-activating mutations are common in BC [55], data have suggested that activation of PIK3CA mutations is not predictive of clinical benefit to mTOR inhibitors. An analysis of primary ER+ BC tumor samples found that high levels of the PIK3CA-G5 gene signature expression (indicative of a PIK3CA mutant phenotype) is indicative of low mTOR-pathway activation [56]. Additionally, an exploratory analysis of the BOLERO-2 study that evaluated the mutational status of key genes (PIK3CA, CCND1 or FGFR1/2) found that, when examining these genes individually, patients whose genes were altered derived similar benefit to everolimus, compared with the overall trial population [57]. Also, patients who had wild-type or a single genetic alteration in the PIK3CA, CCND1, or FGFR1/2 genes seemed to derive greater benefit from everolimus than the overall BOLERO-2 population [57]. Another method to evaluate the potential benefit of mTOR inhibitors is the evaluation of the pattern of protein expression with mTOR pathway activation. In an exploratory translational analysis of the TAMRAD data, high p4EBP, low LKB1 and low PI3K seemed to be associated with everolimus efficacy [58]. However, these data must be validated in larger studies.

Investigational strategies to inhibit PI3K/Akt/mTOR pathway
Additional PI3K/Akt/mTOR pathway inhibitors are being evaluated in clinical trials. For example, NVP-BEZ235 (a dual pan-PI3K/mTOR inhibitor) and BKM120 (a pan-PI3K inhibitor) are being evaluated in combination with letrozole in a Phase I trial in patients with HR+ MBC [203].

Inhibition of the RAS/MAPK pathway
The proto-oncoprotein RAS is a central mediator of many GF receptor-mediated signals, and activation of GF-mediated signaling results in ligand-independent activation of ER-mediated signaling through phosphorylation of ER or its coactivators [99]. Farnesyltransferase inhibitors that inhibit RAS have been shown to synergize with endocrine therapies to inhibit cell growth and induce apoptosis in a preclinical setting [60]. Despite promising preclinical studies, results from Phase II clinical trials evaluating tipifarnib, a farnesyltransferase inhibitor, with tamoxifen [61] or fulvestrant [62] have been disappointing.

Inhibition of Src-Kinase signaling cascade
In preclinical studies, Src-kinase activity was increased in tamoxifen-resistant cells, resulting in increased migration, and inhibition of Src was found to reverse this aggressive phenotype [63] and restore sensitivity to tamoxifen [64]. Dasatinib, an Src-kinase inhibitor, blocked the Src-induced proliferation of tamoxifen-resistant cells [65]. However, in the clinic, dasatinib addition to exemestane in a Phase II trial did not increase PFS, compared with exemestane alone [204]. Nonetheless, several additional Phase II combination studies are ongoing (Table 2).

NF-κB pathway & proteasome inhibition
Active NF-κB in BC tissue identified a high-risk subset of ER+ BC patients [66]. In preclinical studies, inhibition of NF-κB activation with proteasome inhibitors stimulated the growth inhibitory effect of tamoxifen [67,68]. Bortezomib, a proteasome inhibitor that blocks NF-κB activation, was studied in combination with endocrine therapy in a small Phase II trial [69]. Although no clinical response was observed, a Phase II trial in combination with fulvestrant is ongoing (Table 2) [205].

Inhibition of aberrant GF receptor activation
Endocrine resistance has been linked to aberrant expression, activation or signaling through GF RTKs. Aberrant activation of GF RTKs have been shown to activate a number of intracellular signal transduction cascades (Figure 1) [21], including the PI3K/Akt/mTOR, Src-kinase, and RAS/MAPK pathways [70,71]. Hence, a number of GF receptor inhibitors are being studied in clinical trials to overcome endocrine resistance.

HER2 inhibition
Approximately 10% of HR+ BC are also HER2+. [72]. Using archival tumor blocks from the ATAC trial, time to recurrence was shorter in patients with HER2+ BC who were treated with either anastrozole or tamoxifen, suggesting that the effectiveness of tamoxifen might be impeded by HER2 positivity [73]. Additionally, preclinical evidence has suggested that crosstalk between HER2 and ER might lead to endocrine resistance [74,75]. In a Phase III trial comparing letrozole plus lapatinib, a dual HER2 and EGF receptor (EGFR) inhibitor, with letrozole alone as first-line therapy, the combination significantly improved CBR and PFS in postmenopausal women with HER2+ HR+ MBC but not in patients with HR+ HER2+ MBC [76]. Currently, lapatinib in combination with letrozole is FDA approved for patients with HR+, HER2+ MBC [77]. Two trials exploring the efficacy and safety of fulvestrant with lapatinib in patients with previous exposure to endocrine therapy are ongoing (Table 3) [206,207].

Trastuzumab, a HER2 inhibitor, in combination with anastrozole, was studied in a Phase III trial in postmenopausal patients with HER2+ HR+ MBC, some of whom had previous exposure to endocrine therapy [78]. Although the combination significantly
improved PFS by 2.4 months, the PFS for patients receiving the combination was only 4.8 months [78]. A Phase III trial comparing letrozole plus trastuzumab with letrozole alone in patients with HER2+ HR+ MBC, when approximately 50% of patients had received previous tamoxifen therapy, showed that the median TTP with the combination therapy was 14.1 months, compared with 3.3 months with letrozole alone [79]. All together, these observations indicate that combining a HER2 inhibitor with an AI may be clinically effective. A Phase II trial in patients with HR+ HER2– ABC that progressed during previous endocrine therapy is currently evaluating the efficacy of MM-21, an inhibitor of HER3 ligand-stimulated dimerization between HER2 and HER3, in combination with exemestane (Table 3) [208].

EGF-receptor inhibition
Approximately 30% of HR+ BC is EGFR+ [80], and preclinical studies have suggested that EGFR pathway activation may mediate endocrine resistance [81]. In a Phase II trial, CBR was improved with gefitinib, an EGFR inhibitor, in patients with HR+ tamoxifen-resistant tumors, compared with HR+ tamoxifen-resistant tumors [82]. However, results from another Phase II trial failed to support those findings and reported higher toxicity with the combination [83]. In another Phase II trial in patients with HR+ MBC with no previous endocrine therapy for MBC or who progressed on adjuvant tamoxifen therapy, gefitinib in addition to anastrozole significantly improved median PFS by 6.3 months, compared with anastrozole alone [84]; however, another Phase II trial failed to find significant benefit for tamoxifen plus gefitinib [85]. Gefitinib is currently being studied in a Phase II trial in patients with MBC who progressed during first-line endocrine therapy in combination with fulvestrant (Table 3) [209]. Overall, the results for gefitinib addition to endocrine therapy are inconclusive. In addition to gefitinib, vandetanib, a novel tyrosine kinase inhibitor with activity against a number of RTKs, including EGFR and the VEGF receptor (VEGFR) [86], is currently being studied in a Phase II trial in combination with fulvestrant in patients with predominantly bone metastasis HR+ MBC who progressed during previous endocrine therapy (Table 3) [210].

VEGFR inhibition
Preclinical studies have shown that estradiol stimulates proliferation of human endothelial cells and that anti-estrogen inhibits these effects [87]. ER was shown to bind to the promoter of VEGF and activate its transcription, resulting in increased angiogenesis [88]. Retrospective studies of tumor samples have associated higher levels of VEGF with decreased response to endocrine therapy [89]. Bevacizumab, a monoclonal antibody that prevents VEGF and VEGFR interaction, in combination with letrozole, resulted in a median PFS of 17.1 months [90]. The combination of fulvestrant and

<table>
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<tr>
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<th>Enrollment expected (n)</th>
<th>Select primary and secondary outcomes</th>
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<td>NCT01142401</td>
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<td>118</td>
<td>Primary: CBR Secondary: OS, PFS, CBR at 12 and 24 weeks</td>
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AI: Aromatase inhibitor; BC: Breast cancer; BMD: Bone mineral density; CBR: Clinical-benefit rate; MBC: Metastatic breast cancer; ORR: Overall-response rate; OS: Overall survival; PFS: Progression-free survival; TTF: Time to failure.
bevacizumab was tested in a Phase II trial in postmenopausal women with newly diagnosed MBC who were intolerant to an AI or who progressed while receiving an AI [91]. In this same trial, anastrozole plus bevacizumab was studied in patients with earlier stage disease. The median TTP was 21 months with anastrozole plus bevacizumab, essentially as first-line therapy, whereas it was 9 months with fulvestrant and bevacizumab as second-line therapy [91]. Although these results are difficult to interpret, bevacizumab, in combination with endocrine therapy, is currently being tested as a first-line therapy in a Phase III trial (Table 3) [211].

FGF receptor inhibition
Aberrant expression of FGF receptor (FGFR)-1, -2, -3, and -4 have all been shown to result in BC development and to mediate endocrine resistance [92,93]. Brivanib, a dual FGF and VEGF RTK inhibitor, was shown in preclinical studies to inhibit FGF-stimulated growth in FGFR-1-amplified BC cells [94]. Dovitinib, an inhibitor of FGFR, VEGFR, and the PDGF receptor, was shown in a Phase II trial to have some activity in patients who were heavily pretreated [95]. Dovitinib is currently undergoing another Phase II trial in combination with fulvestrant (Table 3) in postmenopausal

Table 3. Ongoing clinical trials using growth factor-receptor inhibitors to overcome endocrine resistance in patients with advanced breast cancer.

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<th>Clinical trial identifier</th>
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<td>Primary: TTP Secondary: CBR, RR</td>
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AI: Aromatase inhibitor; BC: Breast cancer; CBR: Clinical benefit rate; DOR: Duration of response; EGFR: EGF receptor; FGFR: FGF receptor; HR: Hormone receptor; MBC: Metastatic breast cancer; NR: Not reported; NSAI: Nonsteroidal aromatase inhibitor; ORR: Overall-response rate; OS: Overall survival; PFS: Progression-free survival; QoL: Quality of life; RR: Response rate; TTF: Time to failure; TTP: Time to progression; VEGFR: VEGF receptor.
women with endocrine-resistant HR+, HER2- locally advanced breast cancer (ABC) or metastatic breast cancer (MBC) [92,212].

IGF-1 receptor inhibition
Over-activation of IGF-1 receptor signaling has been suggested to mediate endocrine resistance and preclinical data indicate that blocking ER together with IGF1R decreases breast cancer cell proliferation [96]. However, in breast cancer patients whose disease is progressing on previous endocrine therapy, PFS was not improved by a combination of IGF-1 receptor-directed monoclonal antibody and fulvestrant or exemestane, compared with endocrine therapy alone [97].

Epigenetic modulation of signaling through ER
Acetylation is a key component of ER-mediated signaling, and HDACs regulate this process. Abnormal expression of HDACs is associated with many cancers, including breast cancer [98]. Preclinical studies have shown that the antiproliferative activity of tamoxifen is potentiated by HDAC inhibitors [99]. Based on these observations, the HDAC inhibitors are under evaluation [100,101]. Entinostat is an HDAC inhibitor that has been studied in a Phase II trial (ENCORE 301) [101] in combination with exemestane, which showed a trend for prolonged PFS compared with exemestane alone in patients with HR+ MBC progressing during nonsteroidal AI therapy (4.28 vs 2.27 months; hazard ratio: 0.73; 95% CI: 0.50–1.07; p = 0.55). In addition, in an exploratory end point, the entinostat combination improved OS (28.1 months) compared with exemestane alone (19.8 months; hazard ratio: 0.59; 95% CI: 0.36–0.97; p = 0.036) [101].

Targeting cyclin D1: a key cell cycle regulatory protein
Cyclin D1, the regulatory subunit of CDK4 and CDK6, represents a regulatory convergence center for multiple signaling pathways [102]. Cyclin D and its regulation of hyperphosphorylation of retinoblastoma (RB), the tumor suppressor protein, govern cell cycle progression from G1 to S phase [102]. In preclinical breast cell line models of endocrine resistance, dysfunction of the RB pathway, potentially because of deregulation of cyclin D, was associated with luminal B type BCs [103]. Nonfunctional RB pathway was associated with tamoxifen resistance in xenograph models, suggesting that the status of RB pathway may be predictive of endocrine therapy resistance [104]. Molecularly characterized human BC cell lines representing the luminal ER+ subtype were most sensitive to growth inhibition by PD-0332991, a CDK4/6 inhibitor. Additionally, a synergistic interaction between PD-0332991 and tamoxifen was observed [105]. As a result, PD-0332991 was evaluated in combination with letrozole, compared with letrozole alone, in ABC in a Phase II randomized, multicenter trial [106]. The combination was generally well tolerated and resulted in a statistically significant, substantial increase in PFS over letrozole alone with a median PFS of 26.1 months and 7.5 months, respectively (hazard ratio: 0.37; p < 0.001) [106]. A Phase III trial of this promising combination is under way [213].

Conclusion
Endocrine resistance is a major problem in patients with HR- BC. A number of molecular targets that potentially mediate endocrine resistance have been identified and, currently, various strategies utilizing agents that inhibit specific targets identified are being explored. Some of these strategies to overcome endocrine resistance act upstream of ER, while others inhibit the downstream signaling cascades. Many of the strategies are in early stages of clinical trials, whereas others, such as everolimus and HER2-targeted agents have clearly shown clinical benefits combined with endocrine therapy and, consequently, are available for oncologists in clinical practice. A thorough understanding of the various mechanisms of endocrine resistance and current evidence of the clinical usefulness of strategies being explored to overcome resistance, as discussed in this review, are expected to provide clinicians with the much needed insight to manage and improve outcomes in their patients.

Future perspective
Based on a thorough understanding of the various molecular pathways involved in endocrine resistance, many targeted therapies are expected to be developed in the next 5–10 years. Agents with proven activity in ABC, such as everolimus, will move into adjuvant trials. Additionally, predictive biomarkers and biomarkers of efficacy and safety that are currently being explored will help identify patients who may or may not be eligible to receive a specific targeted therapy. It is hoped these developments will enable clinicians to individualize therapy for patients with BC. However, because of the heterogeneity of molecular patterns in patients who become resistant to endocrine therapy and the up-regulation of multiple signaling pathways that have potential therapeutic targets, it might be necessary to target multiple pathways using different agents.

Implications for clinical practice
Despite the wide range of completed and in-progress clinical trials evaluating a large number of new intervention strategies designed to overcome endocrine resistance, in the last few years only two new approaches have received federal drug administration label approval for HR- ABC. These are fulvestrant at a higher 500-mg loading dosage in 2010 and everolimus as an addition to exemestane in 2012.
### Executive summary

**Background**
- Although endocrine therapies are very effective and safe for managing patients with hormone receptor positive (HR+) advanced breast cancer (ABC), endocrine resistance is a significant problem.

**Targeting the estrogen receptor**
- A Phase III trial comparing high-dose fulvestrant to an aromatase inhibitor (AI), as first-line therapy, is ongoing.
- In the second-line setting, low-dose fulvestrant was shown to be as effective as AIs.
- High-dose fulvestrant was shown to be superior to low-dose fulvestrant, resulting in the US FDA approval of high-dose fulvestrant as a second-line therapeutic option for managing postmenopausal women with HR+ metastatic breast cancer (MBC) after disease progression during anti-estrogen therapy.
- Current evidence, based on Phase III trials, on combining low-dose fulvestrant with an AI as first-line therapy in postmenopausal women with HR+ MBC is inconclusive.

**Inhibition of the PI3K/Akt/mTOR pathway**
- Activation of the PI3K/Akt/mTOR pathway mediates endocrine resistance.
- Everolimus, an mTOR inhibitor, in combination with exemestane, was shown to be highly effective in patients with ABC that progressed while on previous nonsteroidal AI therapy, resulting in its approval by the FDA.
- A number of Phase II and III trials evaluating everolimus in combination with endocrine therapy in patients with HR+ ABC are ongoing.
- Sirolimus, another mTOR inhibitor, was shown to be superior in combination with tamoxifen, compared with tamoxifen alone, in a Phase II trial in patients with HR+ MBC.

**Investigational strategies to inhibit the PI3K/Akt/mTOR pathway**
- Clinical trials with dual PI3K-mTOR inhibitors and pan-PI3K inhibitors are ongoing.

**Inhibition of the RAS/MAPK pathway**
- Despite promising preclinical data, clinical trials with tipifarnib, a farnesyltransferase inhibitor, in combination with fulvestrant or tamoxifen in patients with HR+ MBC, have been disappointing.

**Inhibition of Src-kinase signaling cascade**
- Although dasatinib, an Src-kinase inhibitor, in combination with exemestane did not improve progression-free survival, compared with exemestane alone, in patients with HR+ breast cancer refractory to previous nonsteroidal AI therapy, several Phase II trials are ongoing.

**NF-κB pathway & proteasome inhibition**
- No clinical response was observed in a Phase II trial in patients with endocrine therapy-resistant MBC with bortezomib, an inhibitor of NF-κB, in combination with endocrine therapy; however, a Phase II trial in combination with fulvestrant is ongoing.

**HER receptor 2 inhibition**
- Lapatinib, a dual HER2 and EGFR inhibitor, in combination with letrozole, was highly effective as first-line therapy in patients with HER2+, HR- MBC, compared with letrozole alone, resulting in its approval by the FDA.
- Lapatinib is being studied in combination with fulvestrant in patients with previous exposure to endocrine therapy.
- Trastuzumab, a HER2 inhibitor, in combination with letrozole, was effective in patients with HER2+, HR- MBC.
- MM-121, an inhibitor of HER3 ligand-stimulated dimerization of HER2 and HER3, is undergoing clinical trials in combination with exemestane in patients with HR+, HER2- ABC refractory to previous endocrine therapy.

**EGF receptor inhibition**
- Although results from a number of Phase II trials with gefitinib, an EGF receptor inhibitor, have been conflicting, gefitinib is being studied in combination with fulvestrant in patients with MBC that progressed during first-line endocrine therapy.
- Vandetanib, a multitargeted tyrosine kinase with activity against EGF and VEGF receptor, is undergoing a Phase II trial in combination with fulvestrant in patients with predominant bone metastases with HR+ breast cancer that progressed during previous endocrine therapy.

**VEGF receptor inhibition**
- Results from a Phase II trial with bevacizumab, an antibody that blocks the interaction between VEGF and VEGF receptor, in combination with anastrozole, showed that the time to progression was similar to that observed with first-line therapies.
- The improvement in time to progression observed with the combination of bevacizumab with fulvestrant was similar to that observed with second-line therapies.
- Currently, bevacizumab is being studied as a first-line therapy in a Phase III trial in combination with endocrine therapy.
Decisions on how to integrate these approaches into clinical practice is made more difficult by emerging data suggesting that longer duration adjuvant hormone therapy is superior to the usual 5-year regimens [107–109]. Therefore, it is likely that, while fewer postmenopausal BC patients will relapse, among those who relapse, more will be refractory to endocrine therapy. At present, the 500-mg fulvestrant dose is superior to the lower 250-mg fulvestrant dose based upon the data from a Phase III trial in a second-line setting in patients who were resistant to endocrine therapy [31,32]. Conversely, everolimus addition to exemestane is superior to exemestane alone in a Phase III trial [47] given in both cases after prior nonsteroidal AI use.

A sequencing decision for the common situation of prior adjuvant non-steroidal AI treatment between fulvestrant and the everolimus combination requires consideration of therapeutic efficacy and toxicity profiles of both approaches. While the everolimus combination has a higher frequency of side effects, they are generally manageable with appropriate dosage adjustment. The substantial nearly 20% increase in relapse-free survival after only 6 weeks for the everolimus combination in its registry trial demonstrates efficacy against cancers destined for early recurrence [47]. In contrast, fulvestrant is associated with limited side effects and is an injectable rather than an oral therapy. However, the mixed results that have been presented as a first-line therapy using the high- and low-doses [23–26] and the mixed results in combination with aromatase inhibitors [36–38] have caused uncertainty among physicians about how fulvestrant should be used in clinical practice. With further confirmation of previous data, these newer regimens early in the course of ABC are likely to substantially improve the outcome for HR+ BC patients.

Financial & competing interests disclosure
R Chlebowski has been a consultant for Pfizer, Novartis, AstraZeneca, and Amgen and received funding from Novartis and Celgene. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

The author acknowledges writing and editorial assistance by J Sampath and M Graywacke of ApotheCom (Yardley, PA, USA). Funding for the development of this manuscript was provided by Novartis Pharmaceuticals Corporation.

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Papers of special note have been highlighted as:

* of interest
** of considerable interest
In this randomized Phase II study, fulvestrant in a 500-mg schedule improved progression-free survival compared with anastrozole in postmenopausal women with hormone receptor-positive advanced breast cancer.

In this Phase III registry trial, everolimus addition to exemestane was superior to exemestane alone in postmenopausal patients with advanced breast cancer with hormone receptor positive, negative HER 2 disease.


**Endocrine resistance in advanced breast cancer**

**Review: Clinical Trial Outcomes**

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**In this Phase III trial addition of everolimus to trastuzumab and vinorelbine significantly prolonged progression free survival in patients with HER2-positive advanced breast cancer resistant to trastuzumab and pretreated with taxane therapy.**


Review: Clinical Trial Outcomes

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In this randomized Phase II study, PD 0332991, a CDK 4/6 inhibitor plus letrozole demonstrated major improvement in progression-free survival over letrozole alone in postmenopausal women with hormone receptor positive, negative HER2 advanced breast cancer.


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