CLINICAL INVESTIGATION

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

Clin. Invest. (2014) 4(1), 19–33

Rowan T Chlebowski

Chief of Medical Oncology & Hematology, Harbor-UCLA Medical Center, Torrance, CA 90502, USA
Tel.: +1 310 222 2219
Fax: +1 310 320 2564
E-mail: rowanchlebowski@gmail.com

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

FUTURE SCIENCE part of fsg

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 became 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-κB 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

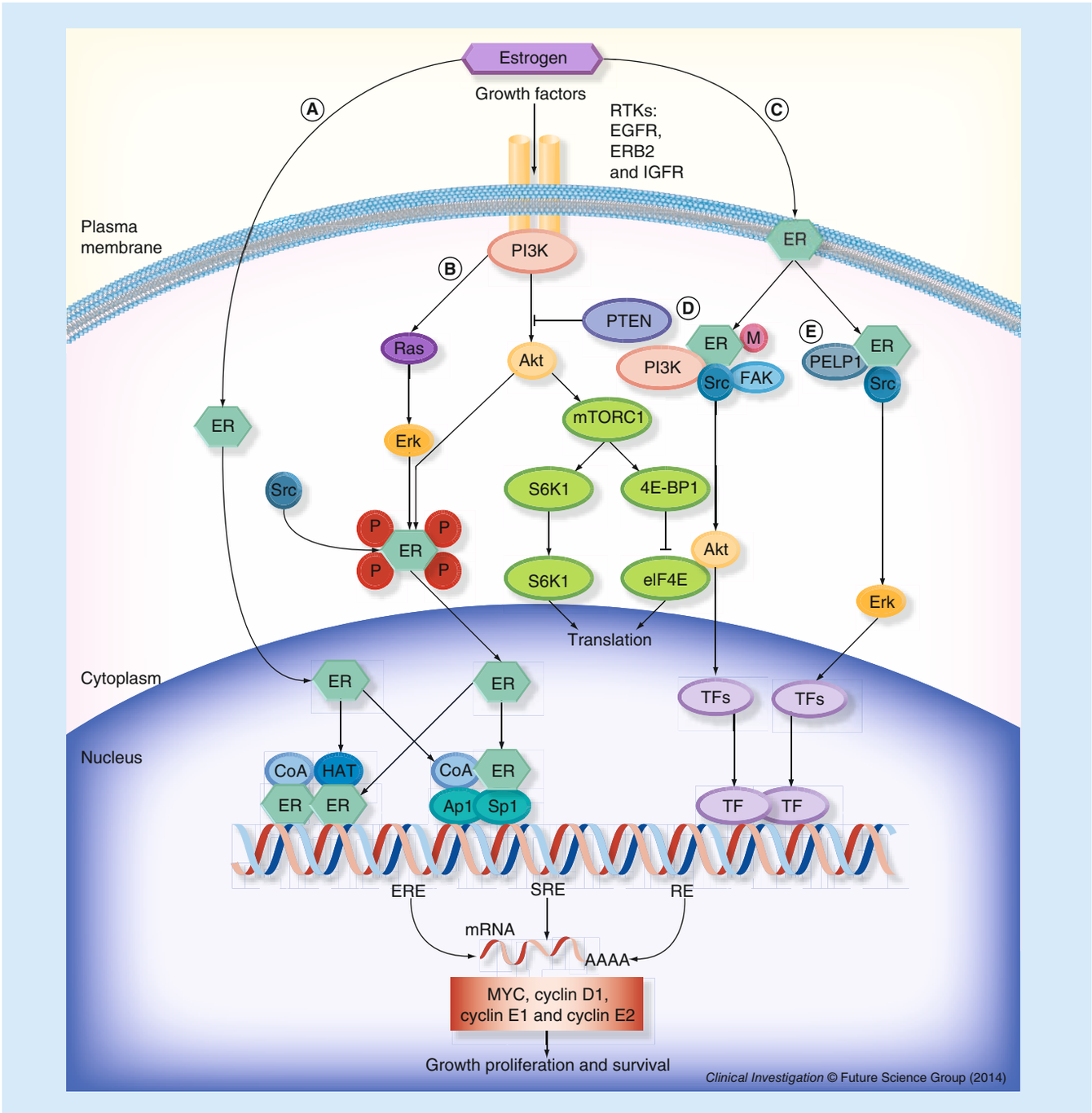

Figure 1. Mechanisms that may mediate endocrine resistance. **(A)** Ligand-bound ER activates gene expression either directly or through protein–protein interactions. **(B)** RTKs such as the EGFR, ERBB2 (also known as HER2) and the IGFR, can activate downstream signaling events, thereby regulating translation 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.
Reprinted with permission from [21].

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-naive, 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.

Clinical trial identifier	Intervention	Patient population	Phase	Expected enrollment (n)	Select primary and secondary outcomes	Ref.
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	Postemenopausal 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; DCR: Disease-control rate; DOR: Duration of response; DRFS: Distant recurrence-free survival; ECOG: Eastern Cooperative Oncology Group; ER: Estrogren 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 PIC3AK-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-GS* 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 [59]. 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 tipifarinib, 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 2. Ongoing clinical trials using other signal transduction inhibitors to overcome endocrine resistance in patients with advanced breast cancer.

Clinical trial identifier	Intervention	Patient population	Phase	Enrollment expected (n)	Select primary and secondary outcomes	Ref.
Src-kinase inhibitors						
NCT00754325	Fulvestrant ± dasatinib	Men and postmenopausal women with advanced BC previously treated with AI	II	100	Primary: PFS Secondary: ORR, TTF, bone markers, toxicity, bone pain, BMD	[223]
NCT00696072	Letrozole ± dasatinib	Postmenopausal women with unresectable, locally recurrent, or MBC – as first- and second-line therapy	II	120	Primary: CBR Secondary: ORR, PFS, TTF, bone markers, toxicity, bone pain, BMD	[224]
NF-κB pathway inhibitors						
NCT01142401	Fulvestrant ± bortezomib	Postmenopausal women with locally advanced BC or MBC resistant to AI therapy	II	118	Primary: CBR Secondary: OS, PFS, CBR at 12 and 24 weeks	[225]

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.

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

Clinical trial identifier	Intervention	Patient population	Phase	Enrollment expected (n)	Select primary and secondary outcomes	Ref.
HER2 inhibitors						
NCT01151046	Exemestane ± MM-121	Postmenopausal women with HR^+ $HER2^-$ locally advanced BC or MBC	II	131	Primary: PFS Secondary: NR	[208]
NCT00390455	Fulvestrant ± lapatinib	Postmenopausal women with HR^+ advanced BC	III	324	Primary: PFS Secondary: OS, DOR, ORR, safety, QoL	[206]
NCT00688194	Fulvestrant ± lapatinib ± AI	Postmenopausal women with MBC who progressed after prior AI therapy	III	396	Primary: PFS Secondary: OS, TTP, RR, CBR	[207]
EGFR inhibitors						
NCT00811369	Fulvestrant ± vandetanib	Postmenopausal women with bone-predominant HR^+ MBC	II	126	Primary: Decrease in bone marker (NTx) Secondary: PFS, response, improvement in pain	[210]
NCT00570258	Fulvestrant ± erlotinib	Patients with HR^+ MBC who progressed on first-line hormonal therapy	II	130	Primary: TTP Secondary: CBR, RR	[209
VEGFR inhibitors						
NCT01466972	Anastrozole or letrozole + pazopanib	Patients with HR^+ advanced BC progressing on NSAI therapy	II	30	Primary: CBR Secondary: TTP, safety	[226]
NCT00545077	Fulvestrant or letrozole ± bevacizumab	Postmenopausal women with advanced BC or MBC; as first-line therapy	III	338	Primary: PFS Secondary: OS, TTF, RR, DOR, clinical benefit proportion, safety	[211]
FGFR inhibitors						
NCT01528345	Fulvestrant ± dovitinib	Postmenopausal women with locally advanced BC or MBC not amenable to curative surgery or radiotherapy and progressing on or after prior endocrine therapy	II	150	Primary: PFS Secondary: OS, ORR, DOR, safety	[212]

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.

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

women with endocrine-resistant HR$^+$, HER2$^-$ locally ABC or 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 along with IFG1R decreases BC cell proliferation [96]. However, in BC 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 BC [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 BC 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 tipifarinib, 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.
- Vandetinib, 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 Grzywacz of ApotheCom (Yardley, PA, USA). Funding for the development of this manuscript was provided by Novartis Pharmaceuticals Corporation.

References

Papers of special note have been highlighted as:

- ▪ of interest
- ▪▪ of considerable interest

1 American Cancer Society. *Cancer facts and figures 2013*. American Cancer Society, Atlanta, GA, USA, 1–62 (2013).

2 Mao C, Yang ZY, He BF *et al.* Toremifene versus tamoxifen for advanced breast cancer. *Cochrane Database Sys. Rev.* 7, CD008926 (2012).

3 American Cancer Society. *Breast Cancer Facts and Figures: 2011–2012*. American Cancer Society, Atlanta, GA, USA, 1–28 (2012).

4 Setiawan VW, Monroe KR, Wilkens LR, Kolonel LN, Pike MC, Henderson BE. Breast cancer risk factors defined by estrogen and progesterone receptor status: the multiethnic cohort study. *Am. J. Epidemiol.* 169(10), 1251–1259 (2009).

5 Miller WR, Larionov AA. Understanding the mechanisms of aromatase inhibitor resistance. *Breast Cancer Res.* 14(1), 201 (2012).

6 Thurlimann B, Robertson JF, Nabholtz JM, Buzdar A, Bonneterre J. Efficacy of tamoxifen following anastrozole ('Arimidex') compared with anastrozole following tamoxifen as first-line treatment for advanced breast cancer in postmenopausal women. *Eur. J. Cancer* 39(16), 2310–2317 (2003).

7 Mouridsen H, Gershanovich M, Sun Y *et al.* Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a Phase III study of the International Letrozole Breast Cancer Group. *J. Clin. Oncol.* 19(10), 2596–2606 (2001).

8 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.* 21(11), 2101–2109 (2003).

9 Paridaens RJ, Dirix LY, Beex LV *et al.* Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: the European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. *J. Clin. Oncol.* 26(30), 4883–4890 (2008).

10 Mauri D, Pavlidis N, Polyzos NP, Ioannidis JP. Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. *J. Natl Cancer Inst.* 98(18), 1285–1291 (2006).

11 Gibson L J, Dawson CK, Lawrence DH, Bliss JM. Aromatase inhibitors for treatment of advanced breast cancer in postmenopausal women. *Cochrane Database Syst. Rev.* 1, CD003370 (2009).

12 Riemsma R, Forbes CA, Kessels A *et al.* Systematic review of aromatase inhibitors in the first-line treatment for hormone sensitive advanced or metastatic breast cancer. *Breast Cancer Res. Treat.* 123(1), 9–24 (2010).

13 Normanno N, Di MM, De ME *et al.* Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer. *Endocr. Rel. Cancer* 12(4), 721–747 (2005).

14 Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. *Ann. Rev. Med.* 62, 233–247 (2011).

▪▪ **Authoritative review of the mechanisms by which breast cancer develop resistance to endocrine therapy.**

15 Chlebowski RT. Changing concepts of hormone receptor-positive advanced breast cancer therapy. *Clin. Breast Cancer* 13, 159–166 (2013).

16 Barrios C, Forbes JF, Jonat W *et al.* The sequential use of endocrine treatment for advanced breast cancer: where are we? *Ann. Oncol.* 23(6), 1378–1386 (2012).

17 Liu MC, Dixon JM, Xuan JJ *et al.* Molecular signaling distinguishes early ER positive breast cancer recurrences despite adjuvant tamoxofen. Presented at: *CTRC-AACR San Antonio Breast Cancer Symposium*. San Antonio, TX, USA, 6–10 December, 2011.

18 Miller WR, Larionov A. Changes in expression of oestrogen regulated and proliferation genes with neoadjuvant treatment highlight heterogeneity of clinical resistance to the aromatase inhibitor, letrozole. *Breast Cancer Res.* 12(4), R52 (2010).

19 Brodie A, Sabnis G. Adaptive changes result in activation of alternate signaling pathways and acquisition of resistance to aromatase inhibitors. *Clin. Cancer Res.* 17(13), 4208–4213 (2011).

20 Xu Y, Sun Q. Headway in resistance to endocrine therapy in breast cancer. *J. Thoracic Dis.* 2(3), 171–177 (2010).

21 Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. *Nat. Rev. Cancer* 9(9), 631–643 (2009).

22 Long X, Nephew KP. Fulvestrant (ICI 182,780)-dependent interacting proteins mediate immobilization and degradation of estrogen receptor-α. *J. Biol. Chem.* 281(14), 9607–9615 (2006).

23 Howell A, Robertson JF, Abram P *et al.* Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial. *J. Clin. Oncol.* 22(9), 1605–1613 (2004).

24 Estevez L, Alvarez I, Tusquets I *et al.* Finding the right dose of fulvestrant in breast cancer. *Cancer Treat. Rev.* 39(2), 136–141 (2013).

25 Robertson JF, Llombart-Cussac A, Rolski J *et al.* Activity of fulvestrant 500mg versus anastrozole 1 mg as first-line treatment for advanced breast cancer: results from the FIRST study. *J. Clin. Oncol.* 27(27), 4530–4535 (2009).

26 Robertson JF, Lindemann JP, Llombart-Cussac A *et al.* Fulvestrant 500 mg versus anastrozole 1 mg for the first-line treatment of advanced breast cancer: follow-up analysis from the randomized 'FIRST' study. *Breast Cancer Res. Treat.* 136(2), 503–511 (2012).

■ **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.**

27 Osborne K, Pippen J, Jones SE *et al.* Double-blind, randomized trial comparing the efficacy and tolerability of fulvestrant versus anastrozole in postmenopausal women with advanced breast cancer progressing on prior endocrine therapy: results of a North American trial. *J. Clin. Oncol.* 20(16), 3386–3395 (2002).

28 Howell A, Robertson JF, Quaresma A J *et al.* Fulvestrant, formerly ICI 182,780, is as effective as anastrozole in postmenopausal women with advanced breast cancer progressing after prior endocrine treatment. *J. Clin. Oncol.* 20(16), 3396–3403 (2002).

29 Flemming J, Madarnas Y, Franek JA. Fulvestrant for systemic therapy of locally advanced or metastatic breast cancer in postmenopausal women: a systematic review. *Breast Cancer Res. Treat.* 115(2), 255–268 (2009).

30 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.* 26(10), 1664–1670 (2008).

31 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.* 28(30), 4594–4600 (2010).

32 di Leo A, Jerusalem G, Petruzelka L *et al.* Final analysis of overall survival for the Phase III CONFIRM trial: fulvestrant 500 mg versus 250 mg. Presented at: *2012 CTRC-AACR San Antonio Breast Cancer Symposium.* San Antonio, TX, USA, 4–8 December, 2012.

33 AstraZeneca. *AstraZeneca updates US label for FASLODEX® (fulvestrant) injection.* AstraZeneca Pharmaceuticals LP, Wilmington, DE, USA (2012).

34 Jelovac D, Macedo L, Goloubeva OG, Handratta V, Brodie AM. Additive antitumor effect of aromatase inhibitor letrozole and antiestrogen fulvestrant in a postmenopausal breast cancer model. *Cancer Res.* 65(12), 5439–5444 (2005).

35 Macedo LF, Sabnis G J, Goloubeva OG, Brodie A. Combination of anastrozole with fulvestrant in the intratumoral aromatase xenograft model. *Cancer Res.* 68(9), 3516–3522 (2008).

36 Mehta RS, Barlow WE, Albain KS *et al.* Combination anastrozole and fulvestrant in metastatic breast cancer. *N. Engl. J. Med.* 367(5), 435–444 (2012).

37 Bergh J, Jonsson PE, Lidbrink EK *et al.* FACT: an open-label randomized phase III study of fulvestrant and anastrozole in combination compared with anastrozole alone as first-line therapy for patients with receptor-positive postmenopausal breast cancer. *J. Clin. Oncol.* 30(16), 1919–1925 (2012).

38 Johnston SR, Kilburn LS, Ellis P *et al.* Fulvestrant plus anastrozole or placebo versus exemestane alone after progression on non-steroidal aromatase inhibitors in postmenopausal patients with hormone-receptor-positive locally advanced or metastatic breast cancer (SoFEA): a composite, multicentre, Phase III randomised trial. *Lancet Oncol.* 14(10), 989–998 (2013).

39 Miller TW, Hennessy BT, Gonzalez-Angulo AM *et al.* Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. *J. Clin. Invest.* 120(7), 2406–2413 (2010).

40 deGraffenried LA, Friedrichs WE, Russell DH *et al.* Inhibition of mTOR activity restores tamoxifen response in breast cancer cells with aberrant Akt Activity. *Clin. Cancer Res.* 10(23), 8059–8067 (2004).

41 Beeram M, Tan QT, Tekmal RR, Russell D, Middleton A, deGraffenried LA. Akt-induced endocrine therapy resistance is reversed by inhibition of mTOR signaling. *Ann. Oncol.* 18(8), 1323–1328 (2007).

42 Bhattacharyya GN, Biswas J, Singh JH *et al.* Reversal of tamoxifen resistance (hormone resistance) by addition of sirolimus (mTOR inhibitor) in metastatic breast cancer. Presented at: *2011 European Multidisciplinary Cancer Congress.* Stockholm, Sweden, 23–27 September 2011.

43 Carpenter J, Roche H, Campone M *et al.* Randomized 3-arm, Phase II study of temsirolimus (CCI-779) in combination with letrozole in postmenopausal women with locally advanced or metastatic breast cancer. Presented at: *The American Society of Clinical Oncology Annual Meeting.* Orlando, FL, USA, 13–17 May, 2005.

44 Wolff AC, Lazar AA, Bondarenko I *et al.* Randomized Phase III placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer. *J. Clin. Oncol.* 31(2), 195–202 (2013).

45 Baselga J, Semiglazov V, van Dam P *et al.* Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. *J. Clin. Oncol.* 27(16), 2630–2637 (2009).

46 Bachelot T, Bourgier C, Cropet C *et al.* Randomized phase II trial of everolimus in combination with tamoxifen in patients with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer with prior exposure to aromatase inhibitors: a GINECO study. *J. Clin. Oncol.* 30(22), 2718–2724 (2012).

47 Baselga J, Campone M, Piccart M *et al.* Everolimus in postmenopausal hormone receptor-positive advanced breast cancer. *N. Engl. J. Med.* 366(6), 520–529 (2012).

■■ **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.**

48 Hortobagyi GN, Piccart M, Rugo H *et al.* Everolimus for postmenopausal women with advanced breast cancer: updated results of the BOLERO 2 Phase III trial. Presented at: *CTRC-AACR San Antonio Breast Cancer Symposium.* San Antonio, TX, USA, 6–10 December 2011.

49 Piccart M, Baselga J, Noguchi S *et al.* Final progression-free survival analysis of BOLERO-2: a Phase III trial of everolimus for postmenopausal women with advanced breast cancer. Presented at: *2012 CTRC-AACR San Antonio Breast Cancer Symposium*. San Antonio, TX, USA, 4–8 December 2012.

50 Novartis Pharmaceuticals Corporation. *Afinitor (everolimus) tablets for oral administration*. East Hanover, NJ, USA (2012).

51 Burris HA III, Lebrun F, Rugo HS *et al.* Health-related quality of life of patients with advanced breast cancer treated with everolimus plus exemestane versus placebo plus exemestane in the Phase III, randomized, controlled, BOLERO-2 trial. *Cancer* 119(10), 1908–1915 (2013).

52 Campone M, Bachelot T, Gnant M *et al.* Effect of visceral metastases on the efficacy and safety of everolimus in postmenopausal women with advanced breast cancer: Subgroup analysis from the BOLERO-2 study. *Eur. J. Cancer* 49, 2621–2632 (2013).

53 Gnant M, Baselga J, Rugo HS *et al.* Effect of everolimus on bone marker levels and progressive disease in bone in BOLERO-2. *J. Natl Cancer Inst.* 105(9), 654–663 (2013).

54 O'Regan RM, Ozgoroglu M, Andre F *et al.* Phase III, randomized, double-blind, placebo-controlled multicenter trial of daily everolimus plus weekly trastuzumab and vinorelbine in trastuzumab-resistant, advanced breast cancer (BOLERO-3). Presented at: *49th Annual Meeting for the American Society for Clinical Oncology.* Chicago, IL, USA, 31 May–4 June 2013.

■■ **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.**

55 Kalinsky K, Jacks LM, Heguy A *et al.* PIK3CA mutation associates with improved outcome in breast cancer. *Clin. Cancer Res.* 15(16), 5049–5059 (2009).

56 Loi S, Michiels S, Baselga J *et al.* PIK3CA genotype and a PIK3CA mutation-related gene signature and response to everolimus and letrozole in estrogen receptor positive breast cancer. *PLoS ONE* 8(1), e53292 (2013).

57 Hortobagyi G, Piccart M, Rugo H *et al.* Genetic alterations and everolimus efficacy in hormone receptor-positive, HER2-negative advanced breast cancer: preliminary correlative results from BOLERO-2. *Presented at: 49th Annual Meeting for the American Society for Clinical Oncology*. Chicago, IL, USA, 31 May–4 June 2013.

58 Treilleux I, Arnedos M, Cropet C *et al.* Predictive markers of everolimus efficacy in hormone receptor-positive metastatic breast cancer: final results of the TAMRAD Trial Translational Study. Presented at: *49th Annual Meeting for the American Society for Clinical Oncology*. Chicago, IL, USA, 31 May–4 June, 2013.

59 Lebowitz PF, Eng-Wong J, Widemann BC *et al.* A Phase I trial and pharmacokinetic study of tipifarnib, a farnesyltransferase inhibitor, and tamoxifen in metastatic breast cancer. *Clin. Cancer Res.* 11(3), 1247–1252 (2005).

60 Ellis CA, Vos MD, Wickline M *et al.* Tamoxifen and the farnesyl transferase inhibitor FTI-277 synergize to inhibit growth in estrogen receptor-positive breast tumor cell lines. *Breast Cancer Res. Treat.* 78(1), 59–67 (2003).

61 Dalenc F, Doisneau-Sixou SF, Allal BC *et al.* Tipifarnib plus tamoxifen in tamoxifen-resistant metastatic breast cancer: a negative Phase II and screening of potential therapeutic markers by proteomic analysis. *Clin. Cancer Res.* 16(4), 1264–1271 (2010).

62 Li T, Christos PJ, Sparano JA *et al.* Phase II trial of the farnesyltransferase inhibitor tipifarnib plus fulvestrant in hormone receptor-positive metastatic breast cancer: New York Cancer Consortium Trial P6205. *Ann. Oncol.* 20(4), 642–647 (2009).

63 Hiscox S, Morgan L, Green TP, Barrow D, Gee J, Nicholson RI. Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells. *Breast Cancer Res. Treat.* 97(3), 263–274 (2006).

64 Hiscox S, Jordan NJ, Smith C *et al.* Dual targeting of Src and ER prevents acquired antihormone resistance in breast cancer cells. *Breast Cancer Res. Treat.* 115(1), 57–67 (2009).

65 Vallabhaneni S, Nair BC, Cortez V *et al.* Significance of ER-Src axis in hormonal therapy resistance. *Breast Cancer Res. Treat.* 130(2), 377–385 (2011).

66 Zhou Y, Eppenberger-Castori S, Marx C *et al.* Activation of nuclear factor-κB (NFkappaB) identifies a high-risk subset of hormone-dependent breast cancers. *Int. J. Biochem. Cell Biol.* 37(5), 1130–1144 (2005).

67 Zhou Y, Eppenberger-Castori S, Eppenberger U, Benz CC. The NFκB pathway and endocrine-resistant breast cancer. *Endocr. Rel. Cancer* 12(Suppl. 1), S37–S46 (2005).

68 de Graffenried LA, Chandrasekar B, Friedrichs WE *et al.* NFκB inhibition markedly enhances sensitivity of resistant breast cancer tumor cells to tamoxifen. *Ann. Oncol.* 15(6), 885–890 (2004).

69 Trinh X B, Sas L, van Laere SJ *et al.* A Phase II study of the combination of endocrine treatment and bortezomib in patients with endocrine-resistant metastatic breast cancer. *Oncol. Rep.* 27(3), 657–663 (2012).

70 Markman B, Dienstmann R, Tabernero J. Targeting the PI3K/Akt/mTOR pathway: beyond rapalogs. *Oncotarget* 1(7), 530–543 (2010).

71 Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat. Rev. Clin. Oncol.* 6(10), 587–595 (2009).

72 Dowsett M, Houghton J, Iden C *et al.* Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. *Ann. Oncol.* 17(5), 818–826 (2006).

73 Dowsett M, Allred C, Knox J *et al.* Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. *J. Clin. Oncol.* 26(7), 1059–1065 (2008).

74 Shou J, Massarweh S, Osborne C K *et al.* Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J. Natl Cancer Inst.* 96(12), 926–935 (2004).

75 Osborne CK, Shou J, Massarweh S, Schiff R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin. Cancer Res.* 11(2 Pt 2), 865s–870s (2005).

76 Johnston S, Pippen J Jr, Pivot X *et al.* Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. *J. Clin. Oncol.* 27(33), 5538–5546 (2009).

77 GlaxoSmithKline, *TYKERB (lapatinib) tablets.* Research Triangle Park, NC, USA (2012).

78 Kaufman B, Mackey JR, Clemens MR *et al.* Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized Phase III TAnDEM study. *J. Clin. Oncol.* 27(33), 5529–5537 (2009).

79 Huober J, Fasching PA, Barsoum M *et al.* Higher efficacy of letrozole in combination with trastuzumab compared to letrozole monotherapy as first-line treatment in patients with HER2-positive, hormone-receptor-positive metastatic breast cancer – results of the eLEcTRA trial. *Breast* 21(1), 27–33 (2012).

80 Klijn JG, Berns PM, Schmitz PI, Foekens JA. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. *Endocr. Rev.* 13(1), 3–17 (1992).

81 Massarweh S, Osborne CK, Creighton C J *et al.* Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function. *Cancer Res.* 68(3), 826–833 (2008).

82 Gutteridge E, Agrawal A, Nicholson R, Leung CK, Robertson J, Gee J. The effects of gefitinib in tamoxifen-resistant and hormone-insensitive breast cancer: a Phase II study. *Int. J. Cancer* 126(8), 1806–1816 (2010).

83 Carlson RW, O'Neill A, Vidaurre T, Gomez HL, Badve SS, Sledge GW. A randomized trial of combination anastrozole plus gefitinib and of combination fulvestrant plus gefitinib in the treatment of postmenopausal women with hormone receptor positive metastatic breast cancer. *Breast Cancer Res. Treat.* 133(3), 1049–1056 (2012).

84 Cristofanilli M, Valero V, Mangalik A *et al.* Phase II, randomized trial to compare anastrozole combined with gefitinib or placebo in postmenopausal women with hormone receptor-positive metastatic breast cancer. *Clin. Cancer Res.*16(6), 1904–1914 (2010).

85 Osborne CK, Neven P, Dirix LY *et al.* Gefitinib or placebo in combination with tamoxifen in patients with hormone receptor positive metastatic breast cancer: a randomized Phase II study. *Clin. Cancer Res.* 17(5), 1147–1159 (2011).

86 Siegfried JM, Gubish CT, Rothstein ME, Henry C, Stabile LP. Combining the multitargeted tyrosine kinase inhibitor vandetanib with the antiestrogen fulvestrant enhances its antitumor effect in non-small cell lung cancer. *J. Thoracic Oncol.* 7(3), 485–495 (2012).

87 Morales DE, McGowan KA, Grant DS *et al.* Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. *Circulation* 91(3), 755–763 (1995).

88 Kazi AA, Jones JM, Koos RD. Chromatin immunoprecipitation analysis of gene expression in the rat uterus in vivo: estrogen-induced recruitment of both estrogen receptor α and hypoxia-inducible factor 1 to the vascular endothelial growth factor promoter. *Mol. Endocrinol.* 19(8), 2006–2019 (2005).

89 Manders P, Beex LV, Tjan-Heijnen VC, Span PN, Sweep CG. Vascular endothelial growth factor is associated with the efficacy of endocrine therapy in patients with advanced breast carcinoma. *Cancer* 98(10), 2125–2132 (2003).

90 Traina TA, Rugo HS, Caravelli JF *et al.* Feasibility trial of letrozole in combination with bevacizumab in patients with metastatic breast cancer. *J. Clin. Oncol.* 28(4), 628–633 (2010).

91 Yardley DA, Burris HA III, Clark BL *et al.* Hormonal therapy plus bevacizumab in postmenopausal patients who have hormone receptor-positive metastatic breast cancer: a Phase II trial of the Sarah Cannon Oncology Research Consortium. *Clin. Breast Cancer* 11(3), 146–152 (2011).

92 Andre F, Greil R, Denduluri N *et al.* Phase II study of the multikinase inhibitor dovitinib (TK1258) or placebo in combination with fulvestrant in postmenopausal, endocrine resistant HER/HR+breast cancer. Presented at: *2012 ASCO Annual Meeting.* Chicago, IL, USA, 1–5 June 2012.

93 Tomlinson DC, Knowles MA, Speirs V. Mechanisms of FGFR3 actions in endocrine resistant breast cancer. *Int. J. Cancer* 130(12), 2857–2866 (2012).

94 Shiang CY, Qi Y, Wang B *et al.* Amplification of fibroblast growth factor receptor-1 in breast cancer and the effects of brivanib alaninate. *Breast Cancer Res. Treat.* 123(3), 747–755 (2010).

95 Andre F, Bachelt TD, Campone M *et al.* A multicenter, open-label Phase II trial of dovitinib, as FGFR1 inhibitor, in FGFR1 amplified and non-amplified metastatic breast cancer. Presented at*: 2011 ASCO Annual Meeting.* 3–7 June, Chicago IL, USA.

96 Cohen BD, Baker DA, Soderstrom C *et al.* Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. *Clin. Cancer Res.* 11(5), 2063–2073 (2005).

97 Robertson JF, Ferrero JM, Bourgeois H *et al.* Ganitumab with either exemestane or fulvestrant for postmenopausal women with advanced, hormone-receptor-positive breast cancer: a randomised, controlled, double-blind, Phase II trial. *Lancet Oncol.* 14(3), 228–235 (2013).

98 Thomas S, Thurn KT, Bicaku E, Marchion DC, Munster PN. Addition of a histone deacetylase inhibitor redirects tamoxifen-treated breast cancer cells into apoptosis, which is opposed by the induction of autophagy. *Breast Cancer Res. Treat.* 130(2), 437–447 (2011).

99 Bicaku E, Marchion DC, Schmitt ML, Munster PN. Selective inhibition of histone deacetylase 2 silences progesterone receptor-mediated signaling. *Cancer Res.* 68(5), 1513–1519 (2008).

100 Munster PN, Thurn KT, Thomas S *et al.* A Phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *Br. J. Cancer* 104(12), 1828–1835 (2011).

101 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.* 31(17), 2128–2135 (2013).

- **In this randomized Phase II study, the histone deacetylase inhibitor entinostat, added to exemestane, improved overall survival, compared with exemestane alone, in postmenopausal woman with advanced hormone receptor positive, negative HER2 disease.**

102 Lange CA, Yee D. Killing the second messenger: targeting loss of cell cycle control in endocrine-resistant breast cancer. *Endocr. Rel. Cancer* 18(4), C19–C24 (2011).

103 Thangavel C, Dean JL, Ertel A *et al.* Therapeutically activating RB: reestablishing cell cycle control in endocrine therapy-resistant breast cancer. *Endocr. Rel. Cancer* 18(3), 333–345 (2011).

104 Lehn S, Ferno M, Jirstrom K, Ryden L, Landberg G. A non-functional retinoblastoma tumor suppressor (RB) pathway in premenopausal breast cancer is associated with resistance to tamoxifen. *Cell Cycle* 10(6), 956–962 (2011).

105 Finn RS, Dering J, Conklin D *et al.* PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res.* 11(5), R77 (2009).

106 Finn RS, Crown JP, Boer K *et al.* Results of a randomized Phase II study of PD0332991, a cyclin-dependent kinase (CDK) 4/6 inhibitor, in combination with letrozole vs letrozole alone for first-line treatment of ER+HER2- advanced breast cancer(BC). *Ann. Oncol.* 23, ii43–ii45 (2012).

- **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.**

107 Jin H, Tu D, Zhao N, Shepherd LE, Goss PE. Longer-term outcomes of letrozole versus placebo after 5 years of tamoxifen in the NCIC CTG MA.17 trial: analyses adjusting for treatment crossover. *J. Clin. Oncol.* 30(7), 718–721 (2012).

108 Davies C, Pan H, Godwin J *et al.* Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet* 381(9869), 805–816 (2013).

109 Gray RG, Rea DW, Handley K *et al.* aTTom (adjuvant Tamoxifen—To offer more?): Randomized trial of 10 versus 5 years of adjuvant tamoxifen among 6,934 women with estrogen receptor-positive (ER+) or ER untested breast cancer – preliminary results. Presented at: *The 2008 ASCO Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008).

Websites

201 National Comprehensive Cancer Network, Inc. NCCN Clinical Practice Guidelines in Oncology. Breast Cancer v2 (2013). www.nccn.org/professionals/physician_gls/pdf/breast.pdf.

202 Clinical Trials database: NCT01674140. www.clinicaltrials.gov/show/NCT01674140

203 Clinical Trials database: NCT01248494. www.clinicaltrials.gov/show/NCT01248494

204 Clinical Trials database: NCT00767520. www.clinicaltrials.gov/show/NCT00767520

205 Clinical Trials database: NCT01142401. www.clinicaltrials.gov/show/NCT01142401

206 Clinical Trials database: NCT00390455. www.clinicaltrials.gov/show/NCT00390455

207 Clinical Trials database: NCT00688194. www.clinicaltrials.gov/show/NCT00688194

208 Clinical Trials database: NCT01151046. www.clinicaltrials.gov/show/NCT01151046

209 Clinical Trials database: NCT00570258. www.clinicaltrials.gov/show NCT00570258

210 Clinical Trials database: NCT00811369. www.clinicaltrials.gov/show/NCT00811369

211 Clinical Trials database: NCT00545077. www.clinicaltrials.gov/show/NCT00545077

212 Clinical Trials database: NCT01528345. www.clinicaltrials.gov/show/NCT01528345

213 Clinical Trials database: NCT01740427. www.clinicaltrials.gov/show/NCT01740427

214 Clinical Trials database: NCT01231659. www.clinicaltrials.gov/show/NCT01231659

215 Clinical Trials database: NCT01499160. www.clinicaltrials.gov/show/NCT01499160

216 Clinical Trials database: NCT01797120. www.clinicaltrials.gov/show/NCT01797120

217 Clinical Trials database: NCT01698918. www.clinicaltrials.gov/show/NCT01698918

218 Clinical Trials database: NCT01437566. www.clinicaltrials.gov/show/NCT01437566

219 Clinical Trials database: NCT01783444. www.clinicaltrials.gov/show/NCT01783444

220 Clinical Trials database: NCT01626222. www.clinicaltrials.gov/show/NCT01626222

221 Clinical Trials database: NCT01633060. www.clinicaltrials.gov/show/NCT01633060

222 Clinical Trials database: NCT01610284. www.clinicaltrials.gov/show/NCT01610284

223 Clinical Trials database: NCT00754325. www.clinicaltrials.gov/show/NCT00754325

224 Clinical Trials database: NCT00696072. www.clinicaltrials.gov/show/NCT00696072

225 Clinical Trials database: NCT01142401. www.clinicaltrials.gov/show/NCT01142401

226 Clinical Trials database: NCT01466972. www.clinicaltrials.gov/show/NCT01466972