Abstract

A greater understanding of the biological mechanisms responsible for de novo and acquired endocrine resistance has led to the rational design of clinical trials exploring the benefit of combining hormonal therapies with novel biological agents in an effort to enhance the efficacy of ER+ breast cancer treatment. These studies are increasingly including parallel biological analyses to elucidate the molecular characteristics of those tumors that are most likely to respond to specific targeted/endocrine combinations in an effort to develop a tailored approach to the management of individual patients. Unfortunately despite encouraging preclinical data, some of these combinations have yielded disappointing results in the clinical setting. This article will review the results of clinical trials of endocrine/biological combinations conducted in early and advanced breast cancer as well as provide an update on ongoing studies.

Introduction

Approximately two-thirds of women with breast cancer have estrogen receptor (ER) positive disease. For several decades, the selective estrogen modulator tamoxifen was offered as standard first line therapy for ER+ breast cancer. In the 1990s, third generation nonsteroidal aromatase inhibitors (AIs) emerged as a useful alternative for post-menopausal women. However, some do not respond (de novo resistance) while others may initially experience meaningful remissions, but eventually progress (acquired resistance). For post menopausal women, further endocrine manipulation may offer some benefit. Options include newer steroidal AIs, such as exemestane, the ER downregulator fulvestrant, or in fact tamoxifen. Women with advanced breast cancer are living longer thanks to an increasing number of available effective chemotherapeutic agents; however, maximizing the benefit of hormonal therapy remains an important priority. Endocrine therapy is well tolerated, oral and can offer clinically meaningful remissions before subsequent relapses that require intravenous cytotoxic agents. Therefore, finding ways to abrogate or delay the onset of endocrine resistance has emerged as an attractive anti-cancer strategy.

Rationale for endocrine/biological combinations

There is increasing evidence that ER+ breast cancer can escape normal endocrine responsiveness by upregulating other signaling pathways involved in cell survival and proliferation. Overexpression of transmembrane peptide growth factor receptors, such as the epidermal growth factor receptor (EGFR) or the human epidermal receptor 2 (HER2) has been associated with poor prognosis and resistance to hormonal therapy [1,2]. Similarly, activation of downstream intracellular signaling via the ras-raf-mitogenic-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway has been implicated in endocrine resistance [3-5]. These pathways activate downstream effectors, which phosphorylate ERα and its co-activators (for example, AIB1), leading to ligand-independent transcription of ER responsive genes [6,7]. For example, both MAPK and Akt have been shown to directly phosphorylate ER, at Ser118 and Ser167, respectively [8,9]. This cross-talk between ER and these alternative signaling pathways may allow cells to escape the antiproliferative effects of endocrine therapy [10,11]. Several biological agents, such as monoclonal antibodies and tyrosine kinase inhibitors, have been developed to target key proteins along these signal transduction cascades.

Importantly, even following the development of endocrine resistance, ER signaling continues to play an important role in the proliferation of breast cancer. Biopsies of tumors from...
inhibitors of the ATP binding site of EGFR and have been Gefitinib and erlotinib are small molecule tyrosine kinase endocrine manipulation with an AI, or fulvestrant [13]. In vitro studies with long-term estrogen-deprived cells (LTED) have shown that breast cancer cells adapt to endocrine deprivation by becoming hypersensitive to estradiol doses as low as $10^{-12}$ M [14,15]. Taken together, these data suggest that ER-mediated signaling remains relevant even in the setting of endocrine resistance. Given the evidence that the ER remains functional and can interact with growth factor signaling pathways, there is a strong rationale for combining novel signal transduction inhibitors with endocrine therapy rather than using them on their own.

Another strategy involves combining hormonal therapy with angiogenesis inhibitors. Angiogenesis, or the formation of new blood vessels, is critical to tumor growth and is principally mediated via tumoral secretion of vascular endothelial growth factor (VEGF). Furthermore, cell line models suggest that estrogen may have pro-angiogenic effects by increasing VEGF gene transcription [16], while increased VEGF receptor signaling may promote endocrine resistance [17]. Co-targeting the tumor with endocrine therapy as well as its associated vasculature using anti-angiogenic agents may provide a more effective anticancer therapy.

A greater understanding of the biological mechanisms responsible for the development of endocrine resistance has led to the rational design of clinical trials exploring the benefit of combining hormonal therapies with novel targeted agents in an effort to enhance the efficacy of ER+ breast cancer treatment. These studies are increasingly including parallel biological analyses to elucidate the molecular characteristics of those tumors that are most likely to respond to specific targeted/endocrine combinations in an effort to develop a tailored approach to the management of individual patients. This article will review the results of clinical trials of endocrine/biological combinations conducted in early and advanced breast cancer as well as provide an update on ongoing studies.

**Combination with EGFR inhibitors**

Gefitinib and erlotinib are small molecule tyrosine kinase inhibitors of the ATP binding site of EGFR and have been shown to delay the development of tamoxifen resistance *in vitro* [18]. Three studies exploring the benefit of combining gefitinib with the aromatase inhibitor anastrozole have been reported (Table 1).

A phase II trial of the combination in women with metastatic breast cancer who had previously failed hormonal therapy showed no clinical activity [19]. In early breast cancer, a randomized neoadjuvant trial of anastrozole alone or in combination with gefitinib given for three months prior to surgery was also negative. In fact, although not statistically significant, there was a trend favoring the endocrine alone arm both in terms of objective response rate (RR = 61% versus 48%, $p=0.067$) and antiproliferative effects as measured by reduction in Ki67 [20]. Further molecular studies of tumor specimens obtained pre- and post-treatment are underway, which may help explain these findings.

In contrast, a preoperative trial by Polychronis and colleagues comparing single-agent gefitinib to gefitinib combined with anastrozole for four to six weeks prior to surgery demonstrated that both treatment arms effectively reduced the size of breast tumors and levels of ER phosphorylation, with the combination treatment outperforming gefitinib alone in terms of reduction in tumor proliferation rate as measured by Ki67 [19]. However, the inclusion criteria for these studies differed. The first two studies showing no benefit to the combination enrolled an unselected population of post-menopausal women with hormone receptor + breast cancer [19,20], while Polychronis and colleagues’ study enrolled only patients who were both ER and EGFR+, thus including only patients expressing the known target for gefitinib, which could explain their positive results [21]. It is worth emphasizing that the reported rates for ER/EGFR co-expression in breast cancer vary quite dramatically among studies (range 5% to 50%) [21-23] and may be attributable to variability in age of archived tissue, fixation techniques and EGFR antibody specificity [24]. However, most studies do support that EGFR and ER expression are inversely related, with twice the rate of EGFR positivity among ER- compared to ER+ tumors [23]. In addition, whether EGFR expression will actually prove to be a valid predictor of response to erlotinib or gefitinib in breast cancer is unclear. Lessons learned from lung cancer suggest that activating mutations in the tyrosine kinase domain or gene amplification may more reliably predict the subset of patients likely to benefit [25,26].

Interim results of a phase II study of erlotinib and letrozole in hormone sensitive metastatic breast cancer were recently reported. The combination was well tolerated and 11 of the first 20 patients experienced clinical benefit rate (CR = 1, partial response (PR) = 4, stable disease (SD) > 6 months = 6) [27]. The trial is ongoing and biomarker analyses are underway to assess whether EGFR, HER2 or ER phosphorylation at Ser118 may predict for benefit from this combination. Planned accrual is 150 patients and the investigators feel that if a clinical benefit of 65% is achieved, a phase III randomized trial would be justified.

Despite these mixed results, a significant number of trials remain ongoing, investigating the combination of gefitinib with AIs or other endocrine therapies such as tamoxifen or the ER downregulator fulvestrant (Table 2). One small study is investigating whether gefitinib may restore endocrine sensitivity by randomizing women with tamoxifen resistant disease to gefitinib alone or in combination with tamoxifen. Interestingly, none of these studies are selecting patients...
Table 1

Combinations of endocrine therapies with biological agents: completed trials in ER+ and/or PgR+ breast cancer

| Clinical setting | Trial phase | Intervention | Clinical endpoints | Biological correlates | Ref. |
|------------------|-------------|--------------|--------------------|-----------------------|------|
| **Combination with gefitinib** | | | | | |
| MBC: hormone-refractory | II (N = 15) | ANA + GEF | PR = 0 SD = 0 | NA | [19] |
| Neoadjuvant PBC | II RCT (N = 188) | ANA versus ANA + GEF for 16 weeks | ORR = 61% versus 48%, \( p = 0.067 \) | Reduction in Ki67 = 83.6% versus 77.4%, \( p = 0.164 \) | [20] |
| Preoperative PBC: EGFR+ only | II RT (N = 56) | GEF versus GEF + ANA for 4-6 weeks | ORR = 50% versus 54% | | [21] |
| **Combination with trastuzumab** | | | | | |
| HER2+ MBC (note: all patients were TRAS and AI naïve, 18% were IHC2+/FISH-) | II (N = 33) | TRAS + LET | PR = 26% SD = 26% | NA | [29] |
| HER2 MBC (all patients were IHC 3+ or FISH+) | III RCT (N = 207) | ANA versus ANA + TRAS | PFS = 2.4 months versus 4.8 months, \( p = 0.0016 \) OS = 23.9 months versus 28.5 months, \( p = 0.325 \) Among 147 evaluable patients: ORR = 6.8% versus 20.3%, \( p = 0.018 \) | | [31] |
| **Combination with lapatinib** | | | | | |
| MBC: included other hormone sensitive advanced cancers | I (N = 17) | LAP + LET | 4 SD including 1 breast cancer | NA | [36] |
| **Combination with farnesyltransferase inhibitors: tipifarnib** | | | | | |
| MBC: hormone resistant | I (N = 12) | TAM + TIP | PR = 2/12 SD >6 months = 1/12 | NA | [42] |
| MBC: tamoxifen resistant | I (N = 20) | TAM + TIP | PR = 1/20 SD >4 months = 6/20 SD >6 months = 4/20 | NA | [41] |
| MBC: tamoxifen resistant | II RCT (N = 120) | LET versus LET + TIP | PR = 38% versus 30% SD = 38% versus 39% | NA | [43] |
| **Combination with mTOR inhibitors: everolimus and temsirolimus** | | | | | |
| MBC: stable or slowly progressing on letrozole. | Ib (N = 9) | LET + EVE 1, 5 or 10 mg daily | No grade 3-4 toxicities at 5 mg (6 patients) or 10 mg (3 patients) | NA | [50] |
| MBC | II (N = 92) | LET versus LET+TEM 10 mg daily versus LET+TEM 30 mg intermittent | ORR = 45% versus 33% versus 40% PFS = 11.6 months versus 11.5 months versus 13.2 months | NA | [51] |
| MBC | III RCT (N = 992) | LET versus LET + TEM intermittent | ORR = 24% versus 24% SD = 19% versus 16% PFS = 9.2 months versus 9.2 months | NA | [52] |
| **Combination with angiogenesis inhibitors: bevacizumab** | | | | | |
| MBC | II (N = 25) | LET + BEV | PR = 2/25 SD >6 months = 13/25 SD <6 months = 4/25 PD = 6/25 | NA | [57] |

AI, aromatase inhibitor; ANA, anastrozole; BEV, bevacizumab; ER, estrogen receptor; EVE, everolimus; FISH, fluorescent in situ hybridization; GEF, gefitinib; HER, human epidermal receptor; IHN, immunohistochemistry; LAP, lapatinib; LET, letrozole; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; NA, none available; ORR, objective response rate (PR + clinical benefit rate); OS, overall survival; PBC, primary breast cancer; PD, progressive disease; pEGFR, phosphorylated endothelial growth factor receptor; pER, phosphorylated ER; PFS, progression free survival; PgR, progesterone; pMAPK, phosphorylated mitogen activated protein kinase; PR, partial response; RCT, randomized controlled trial; RT, randomized trial; SD, stable disease; TAM, tamoxifen; TEM, temsirolimus; TIP, tipifarnib; TRAS, trastuzumab.
| Clinical setting | Trial phase | Intervention | N   | Biological endpoints | NCT protocol # |
|------------------|-------------|--------------|-----|----------------------|----------------|
| **With EGFR inhibitors: gefitinib (GEF) or erlotinib (ERL)** | | | | | |
| Neoadjuvant PBC: HER2+ only | II | GEF x 2 weeks followed by GEF+TAM | 45 | Tumor biomarker analysis at weeks 1, 2, 6 and surgery | 00206492 |
| Neoadjuvant PBC | II | ANA + FUL + GEF | 40 | Tumor biomarker analysis pre- and post-treatment | 00206414 |
| MBC | II RCT | TAM +/- GEF | 274 | Correlate RR to HER2/A1B1 status and other biomarker studies | 00069290 |
| MBC | II RCT | ANA +/- GEF | 174 | Biomarker study | 00077025 |
| MBC | II RCT | ANA +/- GEF | 108 | None specified | 00066378 |
| MBC | II | FUL+GEF | 60 | None specified | 00234403 |
| MBC | II RT | ANA+GEF vs FUL+GEF | 148 | Identify biologic predictors | 00057941 |
| MBC | II | LET + ERL | 150 | Correlation of EGFR, HER2 and pERSer118 to benefit | 00179296 |
| MBC: TAM resistant | II RT | GEF +/- TAM | 46 | Correlate early changes on PET and in plasma DNA to response | 00080743 |
| **With Trastuzumab** | | | | | |
| HER2+ MBC: AI-resistant | II | TRAS monotherapy until PD followed by TRAS + LET | 40 | Correlate benefit with HER2 ECD at baseline and after treatment | 00238290 |
| HER2+ MBC | III RCT | ANA+/ TrAS | NA | None specified | 00022672 |
| HER2+ MBC: TRAS-naive | IV | LET+ TRAS | 370 | None specified | 00171847 |
| **With Lapatinib (LAP)** | | | | | |
| MBC: TAM-resistant | II | LAP+TAM | 41 | None specified | 00118157 |
| MBC | III RCT | LET +/- LAP | 128 | Biomarker and genetic variant analysis | 00073528 |
| MBC: HER2 1+, 2+, 3+ or FISH+ | III RCT | FUL +/- LAP | 324 | None specified | 00390455 |
| **With FTIs: lonafarnib (LON) and tipifarnib (TIP)** | | | | | |
| MBC | II RCT | ANA +/- LON | 124 | None specified | 00081510 |
| MBC | II RCT | ANA +/- LON | 110 | None specified | 00098904 |
| MBC | II RCT | LET+ TIP (continuous) vs LET + TIP (intermittent) | 108 | None specified | 00061971 |
| MBC | II | TAM + TIP | 27-40 | Note: efficacy stratified according to benefit from prior hormone tx | 00052728 |
| MBC | II | FUL + TIP | 45 | Efficacy and toxicity | 00082810 |
| **With mTOR inhibitors: everolimus (EVE) and temsirolimus (TEM)** | | | | | |
| Neoadjuvant PBC | II RCT | LET+/EVE | 255 | None specified | 00107016 |
| MBC | II RCT | LET+/TEM | 90 | None specified | 00061971 |
| **With angiogenesis inhibitors: Bevacizumab (BEV), vatalanib (VAT)** | | | | | |
| Neoadjuvant PBC | II | LET+BEV | 25 | None specified | 00161291 |
| MBC: With acquired endocrine resistance* | II | BEV will be added to current endocrine therapy | 30 | Correlate metabolic response by PET to clinical benefit | 00240071 |
| MBC | II | BEV+LET | 42 | Tumor biomarker analysis Correlate serial endothelial and epithelial cell measurements to response and markers of angiogenesis | 00187694 |
| MBC | II | BEV+ANA or FUL | 80 | None specified | 00405938 |
| MBC | II | VAT+LET | 32 | None specified | 00263198 |
| **With multitargeted TKIs: sorafenib (SOR), imatinib (IMA)** | | | | | |
| MBC: AI-resistant | I/II | ANA +SOR | 50 | Assess changes in Raf and VEGF signaling in tumor and stroma | 00217399 |
| MBC: PDGF or c-kit + only | II | LET+IMA | 45 | Serum measurements of VEGF, bFGF, IL8, PDGF, TNF, E-selectin | 00338728 |
| **Combination with HDAC inhibitor SAHA** | | | | | |
| MBC | II | TAM+SAHA | 42 | Pre-and post treatment ER expression and histone acetylation | 00365599 |

*Acquired endocrine resistance defined as: actively progressing on current endocrine therapy AND history of prior response to current endocrine therapy (i.e. CR, PR or SD > 6 months). PBC, primary breast cancer; MBC, metastatic breast cancer; RCT, randomized controlled trial; RT, randomized trial; PR, partial response; SD, stable disease; ORR, objective response rate (PR+CR); ANA, anastrozole; FUL, fulvestrant; LET, letrozole; PFS, progression free survival; OS, overall survival; TAM, tamoxifen. Source: www.cancer.gov/clinicaltrials.
based on EGFR status, while one neoadjuvant trial of
tamoxifen plus gefitinib is enrolling only HER2+/ER+ patients
based on preclinical evidence that EGFR inhibitors may
reduce HER2 signaling by interfering with EGFR-HER2
heterodimerization [28], and this study includes tumor
biomarker analysis at baseline, weeks 2, 6 and surgery.

**Combinations with trastuzumab**

Trastuzumab, the monoclonal antibody against HER2,
reduces downstream MAPK/ERK1/2 signaling, and at least
partially reverses tamoxifen resistance *in vitro* [2]. It is a
common misconception that most HER2+ tumors are ER-,
and this is illustrated by 50% of patients enrolled in the
recent adjuvant trastuzumab trials being either ER+ or PgR+.
HER2+/ER/PgR+ breast cancer patients therefore represent
an important group with potential *de novo* endocrine
resistance.

A phase II clinical trial of letrozole and trastuzumab in patients
with ER+/HER2+ metastatic breast cancer revealed that the combination was well tolerated and had a clinical benefit rate
(PR + SD) of 50%, with durable objective tumor responses
(PR) for greater than 1 year in 25% of the patients [29]
(Table 1). Inclusion criteria included no prior exposure to
trastuzumab, AI naive and no more than one prior chemo-
therapy for metastatic disease. It is worth noting that the
same clinical benefit rate of 50% is reported with first-line
single agent trastuzumab [30], making it difficult to draw
robust conclusions regarding the added benefit of letrozole.
Furthermore, as highlighted by the investigators, this means
that almost half of these ER+/HER2+ patients demonstrated
*de novo* resistance to dual ER/HER2 inhibition, suggesting
that in a subgroup of patients other targets may be relevant.

The randomized phase III TANDEM trial in 207 patients with
ER+/HER2+ metastatic breast cancer recently reported a
doubling of progression free survival (PFS) with the addition of
trastuzumab over anastrozole alone (4.8 months versus
2.4 months, *p* = 0.0016) [31]. There was no significant
difference in overall survival; however, 70% of patients in the
anastrozole alone arm crossed over to the combination upon
progression. Interestingly, the objective response rate to the
combination was again only 23%, similar to that observed
with first line single agent trastuzumab. Also, the response
rate to single agent anastrozole was surprisingly poor at only
6.8%, although these patients were all ER+/HER2+. A three
arm randomized trial of trastuzumab, an AI or the combination
is required to confirm whether the combination actually offers
an additive benefit.

There remain three ongoing studies looking at trastuzumab in
combination with AIs (Table 2). One of them has an
interesting design - it is enrolling women with trastuzumab
naive HER2+/ER+ metastatic breast cancer who have
progressed after an AI and offering trastuzumab monotherapy
until progression, followed by trastuzumab/letrozole combina-
tion. This study will test the *in vitro* models showing that
growth factor targeted therapy, such as trastuzumab, can
restore endocrine sensitivity.

**Combination with dual EGFR/HER2 inhibitor lapatinib**

Lapatinib is an oral tyrosine kinase inhibitor of both EGFR and
HER2. As a dual inhibitor it may have the potential for greater
anti-tumor effect than strategies targeting a single receptor.
Indeed, EGFR and HER2 belong to a family of four human
EGFRs. These receptors can become aberrantly activated
due to significant receptor overexpression, mutations, or
ligand-dependent receptor dimerization [32,33]. HER2 does
not have a known ligand but represents the preferred
dimerization partner for the other members. A dual
EGFR/HER2 inhibitor may, therefore, have the potential to
target a greater number of tyrosine kinase homo- or hetero-
dimer complexes. *In vitro* data have demonstrated that
estrogen deprivation significantly enhances the anti-
proliferative effects of lapatinib in HER2 amplified breast
cancer cell lines [34,35], which provides the rationale for
investigating the possible additive or synergistic effects of
combining lapatinib with aromatase inhibitors.

A phase I study has shown that the combination with
letrozole is well tolerated, with toxicities consisting mainly of
grade 1-2 diarrhea, nausea, rash and fatigue [36]. Clinical
data to date show that HER2 overexpression is a strong
predictor of response; whether it is in fact an obligate
requirement remains to be determined. Three studies of
lapatinib in combination with endocrine treatment are
ongoing - two are recruiting patients with ER+ disease
regardless of EGFR or HER2 status. The first is a small phase
II trial of lapatinib and tamoxifen in tamoxifen resistant
advanced breast cancer. The design of this study is
supported by preclinical evidence that lapatinib can
significantly enhance sensitivity to tamoxifen in cell lines with
acquired tamoxifen resistance and adaptive HER2
upregulation [35,37]. The second is a large phase III trial
randomizing patients with metastatic ER+ breast cancer to
letrozole +/- lapatinib. Again, patients are selected regardless
of their EGFR/HER2 status, but will be stratified according to
the time interval since adjuvant tamoxifen (>6 or <6 months).
This study may offer important insight into the subgroups of
patients most likely to benefit from a lapatinib-endocrine
combination, such as known HER2+/ER+ breast cancer with
potential _de novo_ endocrine resistance, or tumors that might
develop acquired resistance to letrozole during treatment due
to adaptive HER2 upregulation. To identify the latter, all
patients have serum taken at baseline entry for assessment of
circulating extracellular domain HER2, which has been
reported to be a predictor of poorer outcome with endocrine
therapy, with seroconversion occurring during endocrine
therapy up to 25% of cases [38]. Finally, a CALGB trial is
randomizing women with advanced ER+ breast cancer and
some degree of HER2 expression (for example, fluorescent in
situ hybridization (FISH)+, or immunohistochemistry (IHC) 1+, 2+, or 3+) to fulvestrant alone or in combination with lapatinib.

**Combination with farnesyltransferase inhibitors**

Interfering with the downstream effectors of growth factor receptors has emerged as another effective anti-tumor strategy. Ras proteins are membrane bound GTP-binding proteins that are frequently aberrantly expressed in breast cancer [39]. They act as mitogenic switches between growth factor receptors and downstream intracellular signaling via Raf/MAPK. These proteins require post-translational transfer of a hydrophobic farnesyl moiety to bind to the inner plasma membrane. This reaction is catalyzed by the farnesyltransferase enzyme. Farnesyltransferase inhibitors (FTIs), such as tipifarnib and lonafarnib, were developed in an effort to interrupt this pathway by inhibiting farnesylation, the first step in Ras activation.

Based on encouraging results in cell line and tumor xenograft models [5,40], trials have been conducted in combination with tamoxifen or AIs (Table 1). Interim results on a small study of tamoxifen plus tipifarnib in metastatic tamoxifen resistant breast cancer suggested some activity with 1 PR and 4 SD >6 months among the first 20 patients analyzed [41]. A similar phase I trial reported a CR of 25% with tamoxifen and tipifarnib in a heavily pretreated hormone resistant patient population, further supporting preclinical data that FTIs may modulate endocrine responsiveness [42]. Unfortunately, a larger randomized phase II study of letrozole +/- tipifarnib was disappointing. Among 120 women with tamoxifen resistant disease, the addition of the FTI failed to improve objective response rate (PR = 38% to letrozole alone versus PR = 30% for the combination) [43]. A number of theories could explain these findings. The targets for FTIs are still poorly understood; up to 30 proteins that require farnesylation have been identified to date and their roles in cellular growth and survival are unknown [44]. Furthermore, Ras proteins are not only farnesylated, but can also be modified, or prenylated, by the addition of a geranylgeranyl moiety [44]. Therefore, compensatory geranylgeranylation can offer a way to escape from the inhibitory effects of an FTI. Equally, the benefit of FTIs may be to enhance stable disease and time to disease progression; as such, a much larger randomized phase III trial in over 600 patients may have been needed to demonstrate a significant clinical effect. This phase II study may have been substantially underpowered. Other randomized trials in combination with an aromatase inhibitor as first or second line treatment are ongoing.

**Combination with inhibitors of the mammalian target of rapamycin**

The PI3K/Akt/mammalian target of rapamycin (mTOR) pathway is activated by a number of growth factors, including insulin, insulin-like growth factor I, basic fibroblast growth factor, EGF and VEGF. Inhibiting this key effector of multiple pro-survival signals has, therefore, emerged as a viable therapy. Mutations in the catalytic domain of PI3K have been identified in 20% to 25% of breast cancers [45,46]. A further 15% to 35% of breast cancer patients demonstrate reduced expression of PTEN (phosphatase and tensin homolog deleted on chromosome ten), an endogenous inhibitor of the PI3K/Akt pathway [47]. PTEN loss has been associated with poor prognosis in patients with ER+ breast cancer treated with tamoxifen [48]. Not surprisingly, gain in function mutations in PI3K and loss of PTEN are mutually exclusive. Taken together, this would suggest that half of breast cancers have an upregulated PI3K/Akt pathway due to a constitutively active signal downstream of membrane receptors. This subset of cancers may be resistant to strategies targeting upstream growth factor receptors, but particularly sensitive to PI3K or mTOR inhibition. In addition, preclinical studies have demonstrated that the combination of letrozole with an mTOR inhibitor results in synergistic growth inhibition and apoptosis in ER+ breast cancer cell models [49].

While PI3K inhibitors are still in the early stages of development, mTOR inhibitors are currently being tested in combination with endocrine therapies. A small dose finding phase Ib study of letrozole and the mTOR inhibitor everolimus suggested that the combination is well tolerated [50]. A phase II study of letrozole alone or in combination with another inhibitor, temsirolimus 10 mg daily or 30 mg intermittently (daily for 5 days every 2 weeks), has also been reported [51]. Preliminary results suggested a modest benefit to the combination with intermittent temsirolimus in terms of median PFS (13.2 months versus 11.6 months) and survival (90% versus 76%), although the response rate was not significantly different (40% versus 45%). Notably, the combination was associated with significant toxicities not observed in the letrozole alone arm: grade 1-4 diarrhea (43%) and mucositis (43%) and grade 3-4 hyperglycemia (20%).

Unfortunately, a resulting large phase III randomized trial of letrozole +/- temsirolimus (intermittent schedule) was terminated early after an interim analysis demonstrated a lack of benefit for the combination. Results among the first 992 patients showed no difference in objective response rates or PFS; the combination was better tolerated than reported in the original phase II, with less than 5% grade 3-5 toxicities, including only 4% hyperglycemia [52]. Ongoing trials include a neoadjuvant trial of letrozole +/- everolimus where molecular markers of mTOR inhibition will be correlated with tumor response. In vitro data suggest that mTOR inhibition can lead to a paradoxical increase in Akt activation, via a release of negative feedback on upstream signaling through insulin-like growth factor-1 receptor (IGF1R) [53], or direct activation by the rapamycin-insensitive mTOR-rictor complex [54]. In the setting of hormone receptor positive breast cancer, mTOR inhibition and the resulting increase in Akt
signaling could result in ER phosphorylation on Ser167 and negate the effects of aromatase inhibition, thus limiting the therapeutic benefit of this combination.

Combination with angiogenesis inhibitors

Targeting the vascular supply in addition to the cancer cell itself may be particularly useful against tumors with acquired resistance to traditional therapies. The proof of principle for this approach has been demonstrated by the successful use of a monoclonal antibody against VEGF (bevacizumab) in combination with chemotherapy in the treatment of colon and breast cancers [55,56]. A phase II study of letrozole and bevacizumab reported preliminary results [57]. There were 28 patients evaluable for toxicity. Grade 2-3 side effects consistent with the known safety profile of bevacizumab included hypertension (9 patients), headache (5 patients) and proteinuria (4 patients); there were no grade 4 toxicities. Of the 25 patients assessable for objective responses, there was evidence for modest antitumor activity (PR = 2 patients, SD > 6 months = 13, SD < 6 months = 4 and progressive disease (PD) = 6). Efficacy may have been limited by the fact that previous aromatase inhibitor use without evidence of progression was allowed, and the majority of enrolled patients actually had prior exposure to letrozole or anastrozole. A phase II study of bevacizumab and letrozole in metastatic breast cancer is currently enrolling patients. This trial will correlate tumor and serum molecular markers, including serial circulating endothelial and tumor cell measurements, to response.

The question of whether VEGF inhibition can reverse acquired endocrine resistance is currently being investigated in a phase II trial. Bevacizumab is added to current endocrine therapy in women actively progressing on hormonal therapy, after a prior history of objective response or SD > 6 months. A number of other studies are planned. The CALGB (Cancer and Leukemia Group B) trial 40503 will randomize women with metastatic ER+ breast cancer to first line treatment with letrozole or tamoxifen +/- bevacizumab and will allow for crossover to combination for women progressing in the endocrine therapy alone arm. This design is unique in that it takes into account that women are now receiving a variety of endocrine agents in the adjuvant setting and that there is an increasing need to redefine the role of tamoxifen alone or in combination with targeted agents after relapse on adjuvant AIs. The North Central Cancer Treatment Group (NCCTG) is about to open a trial of first line fulvestrant and bevacizumab in women previously treated with an AI.

Another way to target angiogenesis is using selective inhibitors of the VEGF receptor kinase, such as Vatalanib (PTK787/ZK). This oral small molecule has shown clinical activity in phase I trials, with toxicities consisting mainly of grade 1 or 2 nausea, vomiting, fatigue and dizziness [58] and is currently being investigated in combination with letrozole as first-line treatment for advanced breast cancer.

Combinations with multitargeted tyrosine inhibitors

While most of the biological agents discussed so far aim to target a single receptor or a single family of related kinases, we are also witnessing the development of multitargeted kinase inhibitors. In theory, such an approach may abrogate or delay resistance by disrupting multiple pathways at once. A number of small molecule inhibitors of multiple kinases have entered the clinic. Sorafenib has the potential for both antiangiogenic effects, via inhibition of VEGFR and platelet-derived growth factor receptor (PDGFR), as well as antiproliferative effects, via Raf kinase inhibition. A trial of sorafenib and anastrozole is ongoing and plans to investigate pre- and post-treatment changes in RAF-MAPK and VEGF signaling pathways in tumor and stroma.

Imatinib was one of the first successful targeted therapies, initially developed as a specific inhibitor of the bcr-abl kinase aberrantly expressed in chronic myelogenous leukemia. However, it is now evident that this agent has more than one target, including c-kit and PDGFR, which have been shown to be overexpressed in a subset of breast cancers and associated with poor prognosis [59]. A phase II study of letrozole and imatinib showed that the combination was well tolerated, with grade 3 toxicities consisting of nausea, vomiting and dyspepsia. No efficacy data are available and 9 of the first 15 patients were c-kit and or PDGFR positive [60]. A trial is recruiting patients with PDGFR or c-kit overexpression to letrozole plus imatinib and will include a parallel biomarker analysis.

Combination with modulators of transcription

Another possible mechanism of hormone resistance involves ER post-translational silencing via hypermethylation or histone deacetylation of the ER promoter. Both of these epigenetic modifications can repress ER transcription and have been implicated in endocrine resistance [61]. Importantly, this process has been shown to be reversible in vitro, where treatment of ER negative breast cancer cells with demethylating agents reactivated functional ER expression [62,63]. Histone deacetylation (HDAC) inhibitors are beginning to enter the clinic. Acetylation or the inhibition of deacetylation stabilizes the interaction of nucleosomes and DNA, thereby facilitating transcription of silenced genes [64]. Suberoylanilide hydroxamic acid (SAHA) is an oral HDAC inhibitor that has demonstrated some anti-tumor activity in the phase I setting; dose limiting toxicities included dehydration, anorexia and diarrhea [65].

A study of SAHA in combination with tamoxifen in women previously treated with an AI or tamoxifen is ongoing, the biological rationale for this study being that the HDAC inhibitor may restore endocrine sensitivity by restoring ER expression. Where feasible, serial biopsies will be obtained to assess for treatment induced changes in acetylation and ER expression.
Conclusion
A huge number of endocrine/targeted combinations are currently being tested in the clinic and may offer true promise for the future of women with ER+ breast cancer. However, careful consideration needs to be given both for cross-competing or additive toxicities, and in designing appropriate clinical trials that have the ability to clearly demonstrate any added benefit for these novel (and expensive) therapies.

Subtle deviations from the conventional trial design are required to assess the efficacy of these combinations. Given a relatively favorable toxicity profile, early dose finding studies of endocrine therapy with novel agents should aim to identify the biologically effective dose as defined by demonstration of target inhibition rather than the maximum tolerated dose. As these agents may have more of a cytostatic than cytotoxic effect, alternative endpoints to objective response may be appropriate. Clinical benefit and time to progression are increasingly being used as primary endpoints, while more studies are allowing the inclusion of patients with non-measurable sites of disease. Despite a strong scientific rationale and encouraging results in preclinical studies, some combinations have proven disappointing in the clinical setting. Specific combinations are unlikely to be effective in an unselected population. Taking these combinations forward will require identifying the subset of tumors that demonstrate activation and dependence on the pathways targeted by these novel drugs. An effort to include biomarker studies in future trials remains an imperative. Given the small but statistically significant improvement in disease free survival (DFS) of aromatase inhibitors over tamoxifen, it is not surprising that most ongoing trials are focusing on combining biological agents with Als. However, as more women are receiving an AI upfront in the adjuvant setting, the role of second line tamoxifen alone or in combination with novel therapies remains to be determined, and it is encouraging, therefore, that studies are starting to include tamoxifen.

Competing interests
AF Leary has undertaken preclinical research supported by an independent educational grant from GSK. SRD Johnston as received research funding from AstraZeneca and GlaxoSmithKline. B Sirohi declares that they have no competing interests.

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