Endocrine resistance in hormone-responsive breast cancer: mechanisms and therapeutic strategies

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Abstract
The majority of breast cancers may be considered hormone responsive due to expression of hormone receptors (HR+). Although endocrine therapy is always considered for advanced HR+ breast cancer, the emergence of resistance is inevitable over time and is present from the start in a proportion of patients. In this review, we explore the mechanisms underlying de novo and acquired resistance to endocrine therapy. We comprehensively review newly approved and emerging therapies that have been developed to counteract specific mechanisms of resistance. We discuss the challenges pertinent to this therapeutic arena including the potential relief of negative regulatory feedback inhibition with compensatory pathway activation and the evolution of molecular changes in HR+ breast cancers during treatment. We discuss strategies to address these challenges in order to develop rational therapy approaches for patients with advanced HR+ breast cancer.

Introduction
Approximately 70% of advanced breast cancers are considered ‘hormone responsive’ as defined by expression of the estrogen receptor (ER), progesterone receptor (PR), or both. Various thresholds have been proposed for the level of ER and/or PR immunohistochemical staining required to qualify a breast cancer as hormone responsive, but current guidelines from the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) recommend that these assays be considered positive if at least 1% of tumor nuclei stain for the marker in the setting of appropriate internal and external controls (Hammond et al. 2010). In reality, not all of these tumors with hormone receptor (HR) expression are truly sensitive to manipulation of the ER pathway, with approximately 20% of HR+ metastatic breast cancers (MBC) proving refractory to first-line endocrine therapy (McGuire et al. 1977). Furthermore, the emergence of resistance to endocrine manipulation is inevitable with advanced breast cancer. Although clinicians are encouraged to consider second- and third-line endocrine therapies for patients who initially benefited from first-line treatment (NCCN 2016), the clinical benefit rate declines from approximately 70% for first-line fulvestrant or aromatase inhibitors to around 30% for second or greater lines of therapy (Chia et al. 2008, Ellis et al. 2015). Yet, endocrine therapy can be well tolerated, with durations of response extending into years in some cases. The challenge therefore is to improve our understanding of the mechanisms of endocrine resistance and to develop treatment strategies that utilize this understanding to
extend the duration of effective therapy while minimizing toxicity. In this review, we will discuss the principle mechanisms of endocrine resistance in HR+ breast cancer. We will highlight emerging treatments that have been developed to overcome specific resistance mechanisms and review the results of recently reported studies with these agents. Table 1 summarizes currently enrolling and recently completed phase II/III studies whose results will inform the future selection of therapies for patients with HR+ breast cancer.

**Mechanisms of resistance: ESR1 mutations**

Although loss of ER expression would appear to be a reasonable explanation for the emergence of endocrine resistance, studies of paired primary and metastatic tumor samples indicate that this occurs in only 10% of cases (Sighoko et al. 2014). Similarly, Ellis et al. (2008) found loss of ER expression during neoadjuvant endocrine therapy in less than 10% of patients, and notably in fewer than 20% of non-responders, who would be expected to represent an endocrine-resistant phenotype. Therefore, the estrogen receptor pathway remains a potential target in the majority of cases of endocrine resistance. If loss of ER expression is not the mechanism of resistance in these patients, then what is? We know from studies of patients developing resistance to other ‘oncoreceptor’-targeted therapies such as tyrosine kinase inhibitors of EGFR and c-Kit that resistance is frequently driven by the emergence of additional mutations in the target oncogene (Pao et al. 2005, Tamborini et al. 2006). It is not so surprising, therefore, that a similar molecular mechanism might underpin resistance to therapies targeting the ER. Specifically, attention has recently focused on mutations in the gene ESR1, which encodes ERα (the ER expressed in breast cancer cells). Mutations in ESR1 appear to be rare in a treatment-naive setting and indeed were not identified in a sequencing analysis conducted on 390 ER-positive primary breast cancers (Cancer Genome Atlas Network 2012). However, recent work has revealed that ESR1 mutations become more frequent in metastatic and pretreated ER+ breast cancers. Toy et al. (2013) detected mutations in nine of 36 patients with MBC progressing on endocrine therapy. Highly recurrent mutations were noted at two residues in the ligand-binding domain: replacing tyrosine at residue 537 with serine or asparagine (p.Tyr537Ser/Asn) and replacing aspartic acid at residue 538 with glycine (p.Asp538Gly). The two mutations at the Tyr537 residue had previously been described in studies of small numbers of patients with MBC (Zhang et al. 1997, Li et al. 2013). A schematic representation of the ESR1 gene with the location of the mutations detected (confined to the LBD) is shown in Fig. 1. Sequencing was also performed on primary (untreated) and metastatic (post- aromatase inhibitor treatment) tumors of patients participating in the BOlERO-2 study, and ESR1 mutations were found in 5/44 (11%) tumors in previously treated patients vs 6/183 (3%) tumors in treatment-naïve patients. Robinson et al. (2013) found ESR1 mutations in 6/11 patients with metastatic ER+ breast cancer, all of whom had received antiestrogens and aromatase inhibitors (AIs). For three of these six patients, primary tumor samples were available for comparison and showed no evidence of ESR1 mutation. Taken together, these two studies indicate an ESR1 mutation rate in advanced hormone receptor-positive (HR+) breast cancer of approximately 22%. Similarly, Jeselsohn and colleagues (2014) found a mutation rate of 12% among 76 patients with metastatic ER+ breast cancer, increasing to 20% in a subset with heavily pretreated disease. These mutations have clear therapeutic relevance, as functional analysis in both the Toy and Jeselsohn studies revealed relative resistance to tamoxifen and fulvestrant, which could be partially abrogated with increased dose. Analysis of ESR1 mutations has been performed on archival plasma samples of patients with HR+ MBC and prior nonsteroidal AI treatment participating in two large randomized phase II studies (Fribbens et al. 2016). These analyses indicated mutation rates of 25% among patients with progression on endocrine therapy in the PALOMA3 study (rising to 29% in patients with prior AI therapy) and 39% among patients with prior AI sensitivity in the SoFEA study. The higher rate of mutations in the SoFEA population is in keeping with the existing evidence, indicating that ESR1 mutations are a rare cause of primary endocrine resistance, but emerge more commonly with acquired secondary resistance to AI therapy.

**Mechanisms of resistance: growth factor receptors, PI3K/AKT/mTOR and RAF/MEK/ERK pathway activation**

Overexpression and/or amplification of growth factor receptors including FGFR1, HER2, HER3, EGFR, and IGFR1 are associated with the emergence of endocrine resistance (Ellis et al. 2006, Frogne et al. 2009, Turner et al. 2010, Fox et al. 2011). These growth factor receptor pathways converge on the PI3K/AKT/mTOR and RAF/MEK/ERK pathways.
### Table 1  Recently completed and ongoing randomized studies with endocrine therapy plus/minus targeted therapy in HR+ breast cancer.

<table>
<thead>
<tr>
<th>Study title</th>
<th>Phase</th>
<th>n</th>
<th>Prior therapy</th>
<th>Control arm</th>
<th>Investigational arm</th>
<th>Primary endpoint</th>
<th>Status as of March 1 2016</th>
<th>Clinictrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>FALCON</td>
<td>III</td>
<td>450</td>
<td>No prior endocrine therapy</td>
<td>Anastrozole 1mg daily orally</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter GDC-0810 600mg daily orally</td>
<td>PFS</td>
<td>Completed accrual</td>
<td>NCT01602380</td>
</tr>
<tr>
<td>HydranGea</td>
<td>II</td>
<td>152</td>
<td>Prior AI therapy</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter</td>
<td>Exemestane 25mg daily + everolimus 10mg daily followed at progression by fulvestrant as per control</td>
<td>PFS</td>
<td>Recruiting</td>
<td>NCT02569801</td>
</tr>
<tr>
<td>FEVEX</td>
<td>III</td>
<td>745</td>
<td>Prior NSAI therapy</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter followed at progression by exemestane 25mg daily + everolimus 10mg daily orally</td>
<td>Fulvestrant as per control plus BKM120 100mg daily orally</td>
<td>PFS</td>
<td>Recruiting</td>
<td>NCT01633060</td>
</tr>
<tr>
<td>BELLE-3</td>
<td>III</td>
<td>420</td>
<td>Prior AI and mTORi</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter</td>
<td>Fulvestrant as per control plus taselisib 4mg daily orally</td>
<td>PFS</td>
<td>Recruiting</td>
<td>NCT02340221</td>
</tr>
<tr>
<td>Sandpiper</td>
<td>III</td>
<td>600</td>
<td>Prior AI therapy</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter</td>
<td>Fulvestrant as per control plus alpelisib 300mg daily orally</td>
<td>PFS</td>
<td>Recruiting</td>
<td>NCT02437318</td>
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<tr>
<td>SOLAR-1</td>
<td>III</td>
<td>820</td>
<td>Prior AI therapy</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter</td>
<td>Fulvestrant as per control plus alpelisib 300mg daily orally</td>
<td>PFS</td>
<td>Recruiting</td>
<td>NCT02437318</td>
</tr>
<tr>
<td>PEARL</td>
<td>III</td>
<td>348</td>
<td>Prior NSAI therapy</td>
<td>Capecitabine 1250mg/m² BID orally day 1 – 14, every 21 days</td>
<td>Exemestane 25mg daily orally plus palbociclib 125mg orally day 1 – 21, every 28 days</td>
<td>PFS</td>
<td>Recruiting</td>
<td>NCT02028507</td>
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<tr>
<td>MONALEESA-2</td>
<td>III</td>
<td>667</td>
<td>No prior endocrine therapy</td>
<td>Letrozole 2.5mg daily orally</td>
<td>Letrozole as per control plus ribociclib 600mg orally days 1 – 21, every 28 days</td>
<td>PFS</td>
<td>Completed accrual</td>
<td>NCT01958021</td>
</tr>
<tr>
<td>MONARCH 2</td>
<td>III</td>
<td>630</td>
<td>Prior endocrine therapy</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter</td>
<td>Fulvestrant as per control plus abemaciclib 150mg BID orally</td>
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<td>Completed accrual</td>
<td>NCT02107703</td>
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<tr>
<td></td>
<td>II</td>
<td>97</td>
<td>Prior endocrine therapy</td>
<td>Fulvestrant 500mg IM D1, 14, 28, and every 28 days thereafter</td>
<td>Fulvestrant as per control plus dovitinib 500mg daily (5days on, 2days off)</td>
<td>PFS</td>
<td>Completed accrual</td>
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<tr>
<td></td>
<td>III</td>
<td>600</td>
<td>Prior NSAI therapy</td>
<td>Exemestane 25mg daily orally</td>
<td>Exemestane 25mg daily orally plus entinostat orally days 1, 8, 15, and 22, every 28 days</td>
<td>OS + PFS</td>
<td>Recruiting</td>
<td>NCT02115282</td>
</tr>
</tbody>
</table>

AI, aromatase inhibitor; NSAI, nonsteroidal aromatase inhibitor; mTORi, mTOR inhibitor; IM, intramuscular injection; BID, twice daily.
The PI3K pathway is illustrated in Fig. 2. PI3K is a lipid kinase enzyme belonging to the phosphatidylinositol 3-kinase family (Raynaud et al. 2009). PI3K consists of a regulatory subunit and a catalytic (p110) subunit, which exists in four isoforms: p110α, β, γ, and δ. The PIK3CA gene encodes the p110α isofrom and is mutated in up to 40% of human breast cancers (Campbell et al. 2004, Levine et al. 2005, Saal et al. 2005, Arthur et al. 2014). The frequency of PIK3CA mutations is not increased in metastatic compared with primary breast cancers and there is high concordance between matched primary and recurrent tumor samples for mutation status (Meric-Bernstam et al. 2014). Aberrations in the PI3K intracellular signaling pathway occur in approximately 70% of breast cancers, including the aforementioned PIK3CA mutations; mutation or amplification of other genes encoding isoforms of PI3K subunits; mutations of the downstream effectors AKT1, AKT2, and PDK1; and loss of inhibitory signals from lipid phosphatases PTEN and INPP4B (Fu et al. 2013). PI3K pathway hyperactivation promotes estrogen-independent ER transcriptional activation (Miller et al. 2011b). Conversely, PI3K inhibition results in increased estrogen dependence, providing a rationale for combined endocrine therapy and PI3K inhibition in endocrine-resistant breast cancer (Ghayad et al. 2008, Loi et al. 2010, Miller et al. 2010, Bosch et al. 2015). The mammalian target of rapamycin (mTOR) protein kinase complex is a key downstream effector of the PI3K/AKT pathway existing in two distinct multiprotein complexes, mTORC1 and mTORC2 (Lauring et al. 2013).

Mechanisms of resistance: cell cycle checkpoint alterations

Dysregulated cell cycle progression via alterations of key cell cycle checkpoints can also contribute to loss of endocrine responsiveness (Murphy & Dickler 2015). All cells (both normal and cancerous) receive a plethora of proliferative and antiproliferative signals, the balance of which determines whether a cell will progress from the G1 phase of the cell cycle into the S (synthesis) phase and commit to another cycle of cell division, or withdraw into the quiescent phase (Pardee 1989). Antiproliferative
signals are communicated through the retinoblastoma tumor suppressor protein (pRb) as well as p107 and p130. Rb itself is regulated by complexes of cyclin and cyclin-dependent kinases (CDKs), a family of serine-threonine protein kinases (Morgan 1997). Progression through the G1-S phase requires phosphorylation of Rb by the cyclin-dependent kinase CDK4 (or the highly homologous enzyme CDK6) in complex with cyclin D1, D2, or D3 (Sher 1995). Hyperphosphorylation of Rb reduces its ability to repress activity of the E2F family of transcription factors, leading to increased synthesis of genes whose products are essential for DNA replication and resultant progression to S phase, DNA replication, and mitotic progression (Weinberg 1995). Many tumors increase cyclin D-dependent activity and thereby escape senescence via multiple mechanisms such as p16 inactivation, CDK4 amplification, CDK4 mutation with loss of INK4 binding, cyclin D1 translocation, amplification, or overexpression (Shapiro 2006). Preclinical models suggest a particular role for CDK4/6 inhibition in ER+ breast cancer cells, including estrogen-sensitive and estrogen-resistant models. Cyclin D1 amplification is a common event in ER+ breast cancer, identified in 58% of luminal B cancers and 29% of luminal A cancers (Cancer Genome Atlas Network 2012). Antiestrogen-induced growth arrest in ER+ breast cancer cells is accompanied by decreased cyclin D1 expression, whereas the emergence of endocrine resistance is associated with persistent cyclin D1 expression and Rb phosphorylation (Watts et al. 1995, Thangavel et al. 2011). Evidence of a continued role for CDK4/6 inhibition in endocrine-resistant ER+ cells comes from the evaluation of the CDK4/6 inhibitor palbociclib in vitro in a panel of molecularly characterized breast cancer cell lines that demonstrated most activity in luminal cancers including those with conditioned estrogen resistance (Finn et al. 2009).

Mechanisms of resistance: enhanced autophagy

Macroautophagy (autophagy hereafter) is an intracellular process leading to the degradation of damaged or unnecessary subcellular organelles (Clarke et al. 2015). Along with endoplasmic reticulum stress and the unfolded stress response, autophagy represents a key mechanism by which cancer cells and normal cells deal with various stresses in their microenvironment. In normal cells, it acts at a low level in fed conditions but is up-regulated in the context of stresses such as starvation and hypoxia, whereas cancer cells demonstrate persistent high levels of basal autophagy (White 2012). Inhibition of autophagy has been linked to restoration of endocrine sensitivity and promotion of apoptotic cell death in preclinical models of endocrine-resistant breast cancer (Maycotte & Thorburn 2014). It should be stressed that the pathways involved are complex and feature a high degree of redundancy, leading to differing effects of autophagy inhibition depending on tissue type and context.

New and emerging therapies for HR+ breast cancer

SERDs

The identification of mutations of ESR1 resulting in ligand-independent activation of ERα has reignited interest in a group of agents that result in degradation of ER: the selective estrogen receptor down-regulators (SERDs). Fulvestrant is the first drug in this class and represented a welcome addition to the two other classes of approved breast cancer endocrine therapies (selective estrogen receptor modulators (SERMs) such as tamoxifen and the aromatase inhibitors (AIs) such as anastrozole, letrozole, and exemestane). In contrast to SERMs, SERDs act as ER antagonists without any tissue-specific agonist properties (Wakeling et al. 1991). Binding of fulvestrant to the ligand binding domain (LBD) of ERα results in a conformation incompatible with transcriptional activation (Pike et al. 2001). As noted above, fulvestrant is capable of binding to ERα in the setting of ESR1 LBD mutations, but higher doses are required to achieve target effects. Fulvestrant has a second important effect on ERα, targeting the receptor for proteasomal degradation, leading to its designation as the first-in-class SERD member (Fawell et al. 1990). Importantly, the conformational transcriptional deactivation of ERα appears separate from the ER degrading effect, with the former appearing to be more important in terms of clinical effects (Wardell et al. 2011).

Fulvestrant clinical studies

Fulvestrant was compared with the nonsteroidal aromatase inhibitor anastrozole in the second-line therapy of women with HR+ advanced breast cancer in two phase III studies (Howell et al. 2002, Osborne et al. 2002). The dose of fulvestrant used in these studies was 250mg by intramuscular (IM) injection on day 1, 15, 29, and every 28 days thereafter. Combined analysis (Robertson et al. 2003) indicated non-inferiority of fulvestrant in terms of the primary endpoint of time to progression (TTP). The SoFEA study compared fulvestrant (with or without anastrozole) with the steroidal AI
exemestane in patients with HR+ MBC and evidence of prior endocrine sensitivity in either the adjuvant or advanced setting (Johnston et al. 2013). No difference in the primary endpoint of progression-free survival (PFS) was seen between the three arms. Subsequent prospective–retrospective analysis of ESR1 mutation status in archival plasma circulating tumor DNA (ctDNA) indicated improved outcomes with fulvestrant compared with exemestane among patients with ESR1 mutations, whereas no difference was seen in patients who were ESR1 wild type (Fribbens et al. 2016). These results indicate a potential role for ESR1 mutation status in selecting endocrine therapy in patients with HR+ MBC, ideally with re-evaluation of mutation status at any new ‘decision point’ in the sequence of endocrine therapies.

The phase III CONFIRM study has shown that a higher dose of fulvestrant is superior to the dose utilized in the initial clinical studies, for both disease-free survival (DFS) and overall survival (OS) (Di Leo et al. 2010, Di Leo et al. 2014). The dose revealed to be superior in CONFIRM is 500 mg (or two 250 mg IM injections, one into each buttock) on days 1, 15, 29, and every 28 days thereafter. The open-label randomized phase II FIRST study evaluated this higher 500 mg dose of fulvestrant vs anastrozole in the first-line therapy of ER+ advanced breast cancer. This was a non-inferiority study with a primary endpoint of clinical benefit rate (CBR). It met this endpoint with a CBR of 72.5% vs 67%, respectively, for fulvestrant and anastrozole (Robertson et al. 2009). Subsequent unplanned analyses demonstrated a striking improvement in TTP with fulvestrant (23.4 vs 13.1 months, HR 0.66, \( P = .01 \)) as well as an improvement in OS (Robertson et al. 2012). It should be stressed that this OS analysis comes from a small phase II study and was not planned in the original protocol; nevertheless, it has certainly sparked interest for the outcome of the large double-blind phase III FALCON study (https://www.clinicaltrials.gov/ct2/show/NCT01602380, accessed on 10th January 2016), which has already completed accrual.

The major downside to this agent has been its poor oral bioavailability, requiring intramuscular administration. Furthermore, the degree of ER degradation achieved with fulvestrant appears suboptimal: paired biopsy data indicate that therapy at the initially approved 250 mg dose results in reduction of ERα levels to 50% of baseline, rather than to undetectable levels (Robertson et al. 2001). Even at the higher dose of 500 mg, serial [(18)F] fluoroestradiol PET scans reveal incomplete suppression of estrogen uptake in 38% of patients (van Kruchten et al. 2015). Taken together, these factors indicate a need to develop next-generation SERDs that would not only be orally bioavailable for patient convenience but also exhibit more efficient receptor degradation.

**Newer SERDs**

The recognition that ERα remains a viable target even in the setting of endocrine resistance and the limitations of fulvestrant led to the search for a new generation of orally bioavailable highly selective SERDs. GDC-0810 is one such agent developed through a process of conformation based profiling, followed by refinement and modification (McDonnell et al. 2015). GDC-0810 demonstrated good oral bioavailability and was active in models of tamoxifen-sensitive and tamoxifen-resistant breast cancer (Lai et al. 2015). The phase I component of a phase I/Iia first-in-human study was presented at the American Association for Cancer Research (AACR) Meeting in April 2015 (Dickler et al. 2015). This study enrolled 41 women with ER-positive, HER2-negative advanced breast cancer progressing after at least 6 months of endocrine therapy. ESR1 mutation status was known in 19 patients (46%) and was positive in nine patients. GDC-0810 was administered at total daily doses ranging from 100 to 800 mg, on a once- or twice-daily dosing regimen. Treatment-related adverse events included grade 1–2 diarrhea (63%), fatigue (46%), flatulence (24%), vomiting (22%), and anemia (22%). The recommended phase II dose (RP2D) as a single agent was 600 mg daily with food. At a median follow-up of 8 months, 13 of 31 patients (41%) had stable disease beyond 6 months. Furthermore, two partial responses were seen, both in patients with ESR1 mutations. The phase Iia component of this study is ongoing. GDC-0810 is moving forward in clinical trial development. The randomized phase II HydranGea study will compare GDC0810 with the first-generation SERD, fulvestrant in women with advanced breast cancer resistant to aromatase inhibitors, including a defined subset with ESR1 mutations (https://www.clinicaltrials.gov/ct2/show/NCT02569801, accessed online 10 January 2016). Other agents in clinical trial development include GDC-0927 (SRN-927, Genentech Inc), AZD9496 (AstraZeneca), and LSZ102 (Novartis), which are currently being evaluated in dose-escalating phase I studies in women with ER+, Her2-negative advanced breast cancer (https://clinicaltrials.gov/ct2/show/NCT02316509 and https://clinicaltrials.gov/ct2/show/NCT02248090, accessed 23 February 2016).
mTOR inhibitors

Its position as a key downstream effector of the PI3K/AKT pathway makes mTOR an attractive target for therapies attempting to reverse emerging endocrine resistance. The phase II TAMRAD study evaluated the oral mTOR inhibitor everolimus in combination with tamoxifen vs tamoxifen alone in patients with metastatic ER+, HER2-negative breast cancer progressing after prior AI therapy (Bachelot et al. 2012). There was a significant improvement in the primary endpoint of 6-month CBR, which was 61% in the combination arm vs 42% in the tamoxifen alone arm. Time to progression and OS were also significantly improved with the addition of everolimus. Exploratory analysis suggested the benefit was largely confined to patients with secondary rather than primary endocrine resistance (i.e. patients whose breast cancer relapsed more than 6 months after completing adjuvant AI therapy or progressed after disease control for 6 months or more in the metastatic setting).

In the phase III Bolero 2 trial, everolimus in combination with the steroidal AI exemestane was compared with exemestane alone. This study enrolled patients with advanced ER+ HER2-negative breast cancer and progression after prior nonsteroidal AI therapy. It met its primary endpoint of locally assessed PFS (6.9 vs 2.8 months) and was stopped after an interim analysis (Baselga et al. 2012). The improvement in PFS is impressive but is accompanied by an increase in grade 3–4 toxicities including stomatitis (8%), anemia (6%), dyspnea (4%), fatigue (4%), hyperglycemia (4%), and pneumonitis (3%). The study was not powered to detect a difference in OS and the subsequent analysis of this endpoint was unsurprisingly negative (Piccart et al. 2014). Molecular analysis on archival tumor samples indicated that the benefit of everolimus in BOLERO-2 was independent of PIK3CA mutational status as well as alterations of other closely related genes (PTEN) and pathways (Hortobagyi et al. 2015). Subsequent analysis of cell-free DNA (cfDNA) extracted from archival plasma samples explored the correlation of selected ESR1 and PIK3CA mutations with outcomes in BOLERO-2 (Chandarlapaty et al. 2016, Moynahan et al. 2016). The two ESR1 mutations tested appeared to have differing effects on treatment, as patients with the D538G mutation demonstrated a PFS benefit with the addition of everolimus, while those with the Y537S mutation did not. Allele-specific assays for three hotspot mutations in PIK3CA were consistent with the archival tumor DNA results, indicating similar benefits with the addition of everolimus in PIK3CA-mutant and wild-type tumors. As expected, the selected ESR1 mutations were much more frequently identified in cfDNA vs archival tumor samples (28.4% vs 1.3%), indicating a potential role for this technology in monitoring dynamic tumor genomic changes during treatment.

The improved outcomes seen with the addition of everolimus to AI therapy were not replicated in the phase III HORIZON study that added oral temsirolimus to first-line letrozole (Wolff et al. 2013). Potential explanations for the discrepancy between these two studies include the mTOR inhibitors used (temsirolimus vs everolimus) and differences in the study populations. Of particular note, patients participating in BOLERO-2 had prior AI exposure, whereas patients in HORIZON were AI-naïve and fewer than half had prior adjuvant tamoxifen. Thus, mTOR inhibition may be most useful in the setting of acquired endocrine resistance and may be of less utility in endocrine-responsive disease. The issue of optimal placement of mTOR inhibition in an endocrine therapy sequence is being explored in the phase III FEVEX study, which is enrolling patients with MBC previously treated with nonsteroidal AIs, and randomizing to a sequence of initial fulvestrant followed at progression by the exemestane–everolimus combination, vs the opposite sequence (https://clinicaltrials.gov/ct2/show/NCT02404051, accessed online 10 March 2016).

PI3K inhibitors

With increased understanding of the complexity of intracellular signaling pathways, it has become apparent that inhibition of a given oncoprotein may result in an effect opposite to that intended (i.e. activation of the pathway) through relief of negative upstream feedback inhibition. These negative feedback regulatory mechanisms may be multiple and redundant: for the PI3K/AKT pathway, at least two mechanisms leading to PI3K activation as a consequence of mTOR inhibition have been identified (Chandarlapaty 2012). More proximal blockade (e.g. of PI3K) may obviate some of this feedback activation, improving pathway inhibition. Pictilisib (GDC-0941; Genentech Inc.) is an oral pan-inhibitor of class 1 PI3K (Folkes et al. 2008). The randomized phase II FERGI study enrolled 168 postmenopausal women with advanced ER+ breast cancer who had experienced disease progression on or after aromatase inhibitor therapy (Krop et al. 2014). Patients were randomized 1:1 to fulvestrant...
with or without pictilisib 340 mg daily. The PFS was not statistically improved for the combination arm vs fulvestrant alone in either the intention-to-treat or PIK3CA-mutant populations.

A second oral pan-class 1 PI3K inhibitor, BKM120 (buparlisib, Novartis), demonstrated robust target inhibition in preclinical studies, as well as synergistic activity with cytotoxic agents including taxanes (Maira et al. 2012). It demonstrated promising efficacy in combination with endocrine therapies in two phase I studies, with side effects that were more manageable with an intermittent (5 days on, 2 days off) oral dosing schedule (Mayer et al. 2014, Ma et al. 2015). Common AEs included fatigue, gastrointestinal disorders, transaminitis, hyperglycemia, mood disorders, and maculopapular rash. At the 2015 San Antonio Breast Cancer Symposium, results of the phase III BELLE-2 study were presented (Baselga et al. 2015). This study randomized 1147 women with advanced HR+ HER2-negative breast cancer progressing after prior AI therapy to fulvestrant with buparlisib or placebo. Patients were stratified by PIK3CA mutation status, assessed in archival tumor tissue. There was a modest PFS benefit in the overall study population (6.9 vs 5.0 months, HR 0.78, P<0.001). Among a subset of patients (n=587) assessed for PIK3CA mutations in circulating tumor DNA (ctDNA), the results were more impressive with PFS of 7 vs 3.2 months for the combination vs fulvestrant alone in patients with ctDNA mutations (HR 0.56, P<0.001). No significant difference between the two treatment arms was found among patients with PIK3CA mutations in archival tumor tissue, illustrating the inherent issues in utilizing archival tissue to interrogate dynamically evolving tumors, and the need to assess the current mutational profile whenever possible. Frequent discontinuations due to buparlisib-related adverse events may have limited the efficacy of combination therapy in this trial. Further evaluation of buparlisib is ongoing, including the phase III BELLE-3 study in patients previously treated with everolimus (https://clinicaltrials.gov/ct2/show/NCT01633060, accessed online 10 March 2016).

Selective PI3K inhibitors

Both pictilisib and buparlisib are pan-class 1 PI3K inhibitors (i.e. they inhibit all four p110 isoforms). There may be advantages to more limited isoform selectivity in some tumors, as this may facilitate an increase in the therapeutic drug exposure without increasing off-target side effects. Tumors with activating mutations in the PIK3CA gene would be expected to be sensitive to a drug selectively inhibiting the p110α isoform, whereas PI3K signaling in PTEN-deficient tumors appears more dependent on the p110β isoform and may benefit from pan-class 1 inhibition (Wee et al. 2008, Jiang et al. 2010, Vanhaesebroeck et al. 2010, Schwartz et al. 2015). More selective compounds are entering clinical trials. GDC-0032 (tasilisib; Genentech) is an inhibitor with isoform selectivity for PI3Kα over PI3Kβ (Ndubaku et al. 2013). In a phase II study, tasilisib in combination with fulvestrant demonstrated an ORR of 22% in an endocrine-pretreated population (Dickler et al. 2016). PIK3CA mutations appear promising as a biomarker of efficacy in this study, with an ORR in the PIK3CA-mutant cohort of 38.5% (compared with 10.5% in the wild-type cohort). These results support the stratification by PIK3CA mutation status in the ongoing phase III Sandpiper study evaluating the addition of tasilisib to fulvestrant in patients with prior AI exposure, with a primary endpoint of PFS in PIK3CA-mutant tumors (https://clinicaltrials.gov/ct2/show/NCT02340221, accessed online 18 January 2016). BYL719 (alpelisib; Novartis) is a selective PI3Kα inhibitor equipotent against wild-type and the most common activating mutations of PIK3CA (Fritsch et al. 2014). Not surprisingly, PTEN loss was a negative predictor of sensitivity, given the implication of PI3Kβ in tumor development in a PTEN-deficient setting as outlined above. The phase III SOLAR-1 study is currently accruing postmenopausal women and men with advanced HR+ HER2-negative breast cancer previously treated with AIs (https://clinicaltrials.gov/ct2/show/NCT02437318, accessed online 18 January 2016). Study therapy consists of fulvestrant plus/minus alpelisib. The clinical trial evaluation of alpelisib in triplet regimens with endocrine therapy and CDK4/6 inhibition will be discussed in the next section. Meanwhile, a phase I/II study is evaluating the efficacy of GSK2636771, on oral PI3Kβ isofrom-specific inhibitor in patients with advanced PTEN-deficient solid tumors (Arkenau et al. 2014). This agent has >900-fold selectivity for PI3Kβ over PI3Kα. Preliminary results established an MTD of 400 mg daily, with early signals of antitumor efficacy in this cohort of patients. Toxicity with PI3K inhibitors can occur even with isoform selectivity, representing unwanted ‘on target’ rather than ‘off target’ effects. For example, p110α inhibitors have inevitable effects on glucose homeostasis requiring careful management; development of even more highly selective inhibitors targeting hotspot mutations implicated in tumorigenesis would be advantageous.
CDK4/6 inhibitors

The first drugs developed to target cell cycle progression abnormalities in human cancers were relatively non-selective pan-CDK inhibitors such as flavopiridol, which were limited by off-target effects and complex administration requirements (Sedlacek et al. 1996, Fornier et al. 2007). The therapeutic potential of this strategy in breast cancer was boosted by the development of highly selective inhibitors of CDK4/6 including palbociclib, ribociclib, and abemaciclib. Palbociclib (Ibrance; Pfizer Inc), formerly PD0332991, is the most clinically advanced CDK4/6 inhibitor. Two phase I studies in patients with Rb-positive tumors established a dose of 125 mg daily on a 3 week on, 1 week off schedule as the recommended phase II dose (RP2D) (Schwartz et al. 2011, Flaherty et al. 2012). Myelosuppression (particularly neutropenia) emerged as a common toxicity. The most common non-hematological adverse events were fatigue, diarrhea, nausea, and constipation and were of mainly low grade. Single-agent palbociclib at the RP2D was evaluated in a phase II study in patients with Rb-positive advanced breast cancer (DeMichele et al. 2013). Partial responses were seen in two of 28 (7%) evaluable patients, with stable disease greater than 6 months in 14%. No responses or prolonged disease stabilizations were seen in patients with ER-negative tumors. The disappointing single-agent activity observed in this chemotherapy pretreated population suggests a potential cross-resistance with chemotherapy and increases the attractiveness of combining palbociclib with other agents, notably endocrine therapy.

The multicenter randomized phase I/II PALOMA-1 study evaluated the combination of palbociclib (at 125 mg orally each day on the 3/1 schedule) with letrozole 2.5 mg daily vs letrozole alone in the first-line treatment of ER-positive/HER2-negative advanced breast cancer in postmenopausal women. An initial phase I portion of letrozole plus palbociclib evaluated safety and tolerability of the combination in an unselected population. The phase II component of the study was divided into two parts, the first enrolling patients selected only by ER/HER2 status and the second part enrolling patients further selected for CCND1 amplification and/or p16 loss; 66 patients were enrolled in part 1 and 99 in part 2. Exploratory analysis revealed no additional predictive value of CCND1 status or p16 loss for palbociclib efficacy over ER status alone, and consequently parts 1 and 2 were combined for an overall efficacy analysis. Finn et al. (2015) reported the final PFS efficacy analysis, revealing an impressive improvement in PFS in the combination arm (20.2 vs 10.2 months, \(P=0.0004\)). A significant difference in OS had not yet been seen at this analysis. On Feb 3rd 2015, the FDA granted accelerated approval for palbociclib in combination with letrozole for the first-line treatment of women with ER+, HER2-negative advanced or metastatic breast cancer (US Food and Drugs Administration 2016). The phase III PALOMA-2 study mirrored the phase II component of PALOMA-1, with a randomization between letrozole plus palbociclib vs letrozole alone in a larger patient population of 666 women with advanced, previously untreated ER+, HER2-negative breast cancer (Finn et al. 2016). The results confirmed the improvement in PFS seen in PALOMA-1, with a clinically and statistically significant improvement in investigator assessed PFS from 14.5 to 24.8 months. Neutropenia was more common as expected in the combination arm (80% vs 6%), although neutropenic fever remained uncommon (1.6% vs 0%). These data support the accelerated approval granted to palbociclib in this treatment setting by the FDA based on the PALOMA-1 results.

Meanwhile, the phase III PALOMA-3 study investigated hormonal therapy plus palbociclib in premenopausal and postmenopausal patients whose disease had progressed after prior endocrine therapy. The endocrine therapy utilized in PALOMA-3 was the SERD fulvestrant, with co-administration of the luteinizing hormone releasing hormone (LHRH) analog goserelin in premenopausal patients to induce chemical menopause. Following a pre-planned interim analysis, Turner et al. (2015) reported an improvement in median PFS from 3.8 months in the placebo–fulvestrant arm to 9.2 months in the palbociclib–fulvestrant arm (HR 0.42, 95% CI 0.32, 0.56; \(P<0.001\)). The most common adverse events reported were hematological, with neutropenia occurring in 79% of patients in the palbociclib arm vs 3% in the placebo arm, and leukopenia occurring in 46% vs 4%. Febrile neutropenia was not increased in the palbociclib-containing arm, occurring in 0.6% of patients in both arms. The relatively poor performance of the fulvestrant alone in this study likely reflects the young age and more adverse disease characteristics of the patient population. Nevertheless, the clinically significant improvement in PFS with the addition of palbociclib indicates a key role for CDK4/6 inhibition in overcoming endocrine resistance in the clinical practice. In February 2016, the FDA expanded the approval of palbociclib to include therapy in combination with fulvestrant for HR+, HER2-negative advanced or metastatic breast cancer in women with disease progression following endocrine therapy. The role for a combination of endocrine therapy with CDK4/6
inhibition as an alternative to chemotherapy in breast cancer with emerging endocrine resistance is being put to the test in the PEARL study (https://clinicaltrials.gov/ct2/show/NCT02028507, accessed online 11 January 2016). This ongoing randomized phase III study is comparing palbociclib plus exemestane vs the oral chemotherapy drug capreotabine in patients with advanced ER-positive, HER2-negative metastatic MBC, who have progressed on prior treatment with a nonsteroidal aromatase inhibitor.

Other highly selective CDK4/6 inhibitors including ribociclib and abemaciclib are being evaluated in ER+ breast cancer. Ribociclib (Novartis) demonstrates activity in models of endocrine resistance, including activating aberrations of PIK3CA and/or HER2 (Kim et al. 2013). The phase III Monaleesa-2 study is comparing letrozole plus ribociclib to letrozole alone in women with previously untreated HR+, HER2-negative advanced breast cancer (https://clinicaltrials.gov/ct2/show/NCT01958021, accessed online 11 January 2016). On May 18th 2016, Novartis issued a press release indicating that the independent Data Monitoring Committee had recommended stopping the trial early after a pre-planned interim analysis showed it had met its primary endpoint of a clinically meaningful improvement in PFS with the combination therapy (https://www.novartis.com/news/media-releases/monaleesa-2-trial-novartis-lee011-ribociclib-stopped-due-positive-efficacy, accessed online 25 May 2016). Detailed results of the study are expected to be presented at an international meeting later in 2016. Notable ongoing breast cancer studies include two phase Ib/II studies assessing rational triplet regimens in endocrine-resistant breast cancer: ribociclib in combination with letrozole and the PI3K inhibitor apelisib (http://clinicaltrials.gov/ct2/show/NCT01872260, accessed online 11 January 2016), and in combination with the steroidal aromatase inhibitor exemestane and the mTOR inhibitor everolimus (http://clinicaltrials.gov/ct2/show/NCT01857193, accessed online 23 February 2016). These combinations are attractive given preclinical evidence that CDK4/6 inhibition may overcome intrinsic and adaptive resistance to PI3K inhibition (Vora et al. 2014). Preliminary data from 16 patients enrolled in the exemestane/everolimus/ribociclib combination study indicate that the triplet combination appears feasible (Bardia et al. 2014). The dose expansion phase will further explore tolerability of the triplet in patients naïve to CDK4/6 therapy and of the exemestane/ribociclib doublet in patients with prior CDK4/6 inhibitor therapy. Similarly, phase I data for the doublet combinations of ribociclib/letrozole and ribociclib/apelisib have been presented (Munster et al. 2014). Both arms demonstrated acceptable safety profiles, with neutropenia observed as expected with ribociclib. Accrual to the third arm (ribociclib, apelisib plus letrozole) will proceed, followed by a planned phase 2 randomization between the triplet and the two doublets.

Abemaciclib (Eli Lilly) was evaluated in a phase I study across multiple tumor types, including a cohort of 47 women with heavily pretreated metastatic breast cancer. Tumor responses were confined to women with ER+ disease, with a response rate of 25% (nine of 36) in this subset (Patnaik et al. 2014a). The disease control rate (defined for this study as partial responses plus stable disease of any duration) was 81% in the ER+ subset. Neutropenia was the only grade 3/4 adverse event seen in more than 5% of patients, occurring in 11%. A separate MBC cohort (n=13) evaluated the combination of abemaciclib 200mg twice daily plus fulvestrant (Patnaik et al. 2014b). The most common possibly treatment-related AEs were diarrhea (8% G3), fatigue, neutropenia, nausea, vomiting, and leukopenia. There were no episodes of febrile neutropenia. Of these, eight confirmed and three unconfirmed partial responses were observed. Ongoing studies are assessing abemaciclib in the HR+ breast cancer population, both as monotherapy and in combination with endocrine agents. The phase II MONARCH1 study evaluated continuous oral dosing of abemaciclib (200mg twice daily) in patients with HR+ MBC who had received prior endocrine therapy and one to two prior chemotherapy regimens, including taxanes (Dickler et al. 2016). The ORR was 19.7%, which is similar to that seen with single-agent chemotherapy in this setting, with a median duration of response of 8.6 months. Diarrhea was a common adverse event, occurring at grade 3 in 19.7% of patients. This was commonly seen early in therapy and resolved quickly with therapy interruption, antidiarrheal agents, and dose reduction. The phase III MONARCH2 study is comparing the combination of fulvestrant plus continuous daily oral abemaciclib vs fulvestrant alone in patients with advanced breast cancer and prior endocrine therapy (http://clinicaltrials.gov/ct2/show/NCT02107703. Accessed online 11 January 2016).

Other therapies: inhibitors of FGFR, IGF1R, HDAC, and autophagy

Dovitinib is an oral multi-targeting tyrosine kinase inhibitor that includes fibroblast growth factor (FGFR) 1–3 among its targets. A phase II trial evaluated dovitinib in 81 women with HR+ and HR-negative metastatic
breast cancer; unconfirmed responses and stable disease >6 months were seen in 25% of HR+ tumors with FGFR1 amplification compared with 3% of patients with HR-negative FGFR1 non-amplified disease (André et al. 2013). Current studies include a randomized phase II study evaluating fulvestrant plus/minus dovitinib in patients with prior endocrine therapy (https://www.clinicaltrials.gov/ct2/show/NCT01528345, accessed online 28 February 2016) and a phase I/II study adding dovitinib to aromatase inhibitor therapy in patients experiencing progression on aromatase inhibitor monotherapy (André & Cortés 2015). Molecular pre-screening to test for FGFR pathway activation is included in the eligibility criteria for these studies. Ongoing studies are evaluating additional FGFR targeting agents AZD4547, lucitaniib, BGJ398, and JNJ-42756493 in breast cancer (https://www.clinicaltrials.gov/ct2/results?term=%22FGFR%22+AND%22breast+cancer%22&recr=Open, accessed online 28 February 2016).

Inhibition of insulin-like growth factor receptor 1 (IGFR1) with the monoclonal antibody AMG-479 (ganitumumab) has been tested in a randomized phase II study in endocrine-pretreated HR+ breast cancer, with no improvement in PFS (Robertson et al. 2013). The oral inhibitor of histone deacetylase (HDAC) entinostat in combination with exemestane prolonged DFS and OS over exemestane alone in a randomized phase II study in patients with prior nonsteroidal AI therapy (Yardley et al. 2013). A randomized phase III study is currently accruing to compare entinostat and exemestane to the endocrine therapy alone in patients with advanced HR+ breast cancer and prior nonsteroidal AI treatment (https://www.clinicaltrials.gov/ct2/show/NCT02115282, accessed online 19 Jan 2016).

The antimalarial drug chloroquine and antilupus drug hydroxychloroquine are known to be inhibitors of autophagy and are currently being explored in early phase trials in breast cancer. In a double-blind randomized phase II ‘window’ trial, patients are receiving to 2–6 weeks of chloroquine treatment leading up to surgery for their breast cancer, with outcomes including effects on tumor proliferation and apoptosis being measured (https://www.clinicaltrials.gov/ct2/show/NCT02333890, accessed online 23 May 2016). In a similar preoperative window setting, the PINC study is examining the effect of neoadjuvant chloroquine on the ability of ductal carcinoma in situ (DCIS) to survive and invade (https://www.clinicaltrials.gov/ct2/show/NCT01023477, accessed online 23 May 2016). Meanwhile, in the advanced endocrine-resistant breast cancer setting, hydroxychloroquine is being evaluated in combination with endocrine therapy in a phase Ib/II study (https://www.clinicaltrials.gov/ct2/show/NCT02414776, accessed online 23 May 2016).

Conclusion

Endocrine therapy can provide prolonged disease control in HR+ advanced breast cancer, and strategies to prolong the utility of endocrine therapy are highly desirable. Although our understanding of the mechanisms underlying the emergence of endocrine resistance has improved greatly, the results of clinical trials have been mixed. Notable successes include the FDA approval of agents targeting mTOR and CDK4/6 in combination with endocrine therapy, whereas failures include the disappointing performance of pan-PI3K inhibition with pictilisib. Increasing target selectivity may improve efficacy while decreasing off-target toxicity, as has been seen with newer CDK4/6 inhibitors compared with earlier pan-CDK inhibitors. It is hoped that similar enhanced clinical utility will be seen with isoform-selective PI3K inhibitors. Similarly, efforts to develop next-generation SERDs combining improved oral bioavailability with enhanced ER targeting appear promising.

An additional approach to overcome endocrine resistance is to target multiple intracellular pathways and/or multiple points within a pathway. This approach tackles the built-in redundancy within signaling pathways and attempts to prevent iatrogenic release of regulatory feedback inhibition with compensatory pathway stimulation. It does carry the risk of increased toxicity. This strategy is being explored in current trials exploring the tolerability and efficacy of triplet regimens (including antiestrogen/mTOR/CDK inhibitor and antiestrogen/PI3K/CDK inhibitor combinations) in advanced breast cancer.

Finally, it is imperative that we continue to strive to understand the dynamic changes that take place over the course of treatment in individual cancers. The recognition of the emergence of ESR1 mutations during endocrine therapy underlines the importance of repeated sampling of tumors over time, whether that be with tumor tissue biopsies or ‘liquid biopsies’, i.e. circulating tumor cells and circulating DNA. Tumor heterogeneity may exist and/or develop over time, such that ‘liquid biopsies’ may better represent the molecular alterations underlying the biology of the cancer. Validation studies are underway to compare these two approaches. Analyses of tumor and cfDNA samples from patients participating in clinical trials are beginning to create a landscape where therapy
selection for individual patients may be assisted by interrogating the current mutational profile of the tumor. Patients with ESR1 mutations may be better served by a SERD than an AI, for example while PIK3CA mutations do not appear to affect mTOR inhibitor therapy efficacy but appear to be a biomarker for PI3K inhibitor efficacy. Continued collection of tissue and plasma samples from patients participating in clinical trials is vital in order to refine a dynamic genomic-based approach to therapy selection. Such an approach will bring endocrine therapy for breast cancer closer to the ultimate goal of personalized medicine: getting ‘the right drug for the right patient at the right dose and time’ (Sadee & Dai 2005).

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References
Di Leo A, Jerusalem G, Petruzela L, Torres R, Bondarenko IN, Khasanov R, Verhoeven D, Pedrini JL, Smirnova I, Lichinir MR,
et al. 2014 Final overall survival: fulvestrant 500 mg vs 250 mg in the randomized CONFIRM trial. Journal of the National Cancer Institute **106**(3) 337. (doi:10.1093/jnci/djt337)


Dickler MN, Saura C, Richards DA, Krop IE, Cervantes A, Bedard PL, Patel MR, Pusztai L, Oliveria M, Ware JA, et al. 2016 A phase II study of the PI3K inhibitor taselisib (GDC-0032) combined with fulvestrant (F) in patients (pts) with HER2-negative (HER2-), hormone receptor-positive (HR+) advanced breast cancer (BC). Journal of Clinical Oncology **34** (Supplement) abstract S20. (available at: http://meetinglibrary.asco.org/content/165518-176)


Wardell SE, Marks JR & McDonnell DP 2011 The turnover of estrogen receptor α by the selective estrogen receptor degrader (SERD) fulvestrant is a saturable process that is not required for antagonist efficacy. Biochemical Pharmacology 82:122–130. (doi:10.1016/j.bcp.2011.03.031)


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