

WJCO 5th Anniversary Special Issues (2): Breast cancer**Mechanisms and therapeutic advances in the management of endocrine-resistant breast cancer**

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Abstract

The estrogen receptor (ER) pathway plays a critical role in breast cancer development and progression. Endocrine therapy targeting estrogen action is the most important systemic therapy for ER positive breast cancer. However its efficacy is limited by intrinsic and acquired resistance. Mechanisms responsible for endocrine resistance include deregulation of the ER pathway itself, including loss of ER expression, post-translational modification of ER, deregulation of ER co-activators; increased receptor tyrosine kinase signaling leading to activation of various intracellular pathways involved in signal transduction, proliferation and cell survival, including growth factor receptor tyrosine kinases human epidermal growth factor receptor-2, epidermal growth factor receptor, PI3K/AKT/mammalian target of rapamycin (mTOR), Mitogen activated kinase (MAPK)/ERK, fibroblast growth factor receptor, insulin-like growth factor-1 receptor; alterations in cell cycle and apoptotic machinery; Epigenetic modification

including dysregulation of DNA methylation, histone modification, and nucleosome remodeling; and altered expression of specific microRNAs. Functional genomics has helped us identify a catalog of genetic and epigenetic alterations that may be exploited as potential therapeutic targets and biomarkers of response. New treatment combinations targeting ER and such oncogenic signaling pathways which block the crosstalk between these pathways have been proven effective in preclinical models. Results of recent clinical studies suggest that subsets of patients benefit from the combination of inhibitor targeting certain oncogenic signaling pathway with endocrine therapy. Especially, inhibition of the mTOR signaling pathway, a key component implicated in mediating multiple signaling cascades, offers a promising approach to restore sensitivity to endocrine therapy in breast cancer. We systematically reviewed important publications cited in PubMed, recent abstracts from ASCO annual meetings and San Antonio Breast Cancer Symposium, and relevant trials registered at ClinicalTrials.gov. We present the molecular mechanisms contributing to endocrine resistance, in particular focusing on the biological rationale for the clinical development of novel targeted agents in endocrine resistant breast cancer. We summarize clinical trials utilizing novel strategies to overcome therapeutic resistance, highlighting the need to better identify the appropriate patients whose diseases are most likely to benefit from these specific strategies.

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Key words: Endocrine therapy; Endocrine resistance; Breast cancer; Therapeutic advances; Targeted therapy

Core tip: Endocrine therapy is the important systemic therapy for hormone receptor positive breast cancer. However, treatment resistance is common. Multiple mechanisms responsible for endocrine resistance have been identified over the past decade. New treatment

combinations targeting estrogen receptor and growth factor receptor signaling which block the crosstalk between these pathways are effective in preclinical models and clinical studies. In this review, we summarize the complex genomic and epigenetic regulatory pathways involved in endocrine resistance, in particular focusing on the clinical trials utilizing novel strategies to overcome therapeutic resistance.

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INTRODUCTION

Estrogen receptor (ER) is expressed in about 75% of human breast cancers which is the one of the leading cause of death for women globally. The estrogen-bound ER functions through ligand-activated transcriptional regulation (genomic actions) and by acting as a component of signaling cascades outside of the nucleus (non-genomic actions)^[1-4]. Clinical observations and laboratory studies suggest ER signaling pathway is the major driver in promoting proliferation, survival and invasion of ER-positive breast cancer cells^[5]. Endocrine therapy is the mainstay of treatment for patients with ER-positive breast cancer, especially those with metastatic disease. Endocrine therapies include treatments which target ER by blocking receptor binding with an antagonist or by depriving the tumor of estrogen. The three broad groups of currently approved anti-estrogen therapies are selective estrogen receptor modulators (SERMs) such as tamoxifen, raloxifene and toremifene, which block activity of ER; selective estrogen receptor down regulators (SERDs) such as fulvestrant, which induce destabilization and degradation of ER; and aromatase inhibitors (AIs), including steroidal/irreversible (anastrozole and letrozole) and nonsteroidal/reversible (exemestane) inhibitors, which decrease estrogen production in peripheral tissues and within the tumors through inhibition of the enzyme aromatase^[5-11]. Endocrine therapy as the first targeted therapy in cancer treatment has successfully improved outcome of millions of breast cancer patients in the past 30 years^[5,12].

There is evidence that some breast tumors are more resistant to endocrine therapy than others, despite expressing ER. This is supported by stratification of ER positive tumors into luminal A and luminal B subtypes based on molecular profiling studies over the last decade. The luminal B subtype is more aggressive and less endocrine sensitive, while the luminal A subtype is more indolent and endocrine responsive^[13-15]. Recently The Cancer Genome Atlas (TCGA) data reinforces that luminal B cancers represent a unique subtype of breast cancer, with a distinctive biology from that of luminal A cancers. Multigene tests performed on the primary breast

tumor are increasingly utilized in clinical practice to assist in adjuvant therapy decision making and to distinguish which patients might benefit most from a combination of endocrine therapy plus chemotherapy, rather than endocrine therapy alone. For example, the 21-gene (OncotypeDx) and 70-gene (MammaPrint) assays can classify ER positive tumors according to their aggressiveness, risk of recurrence, and likelihood of benefitting from adjuvant endocrine or chemotherapy. PAM50 is a 50 gene expression assay to separate breast tumor samples into known intrinsic molecular subtypes (basal-like, HER-2 enriched, luminal A and luminal B) and correlate with risk of relapse. The progesterone receptor (PR) is expressed in half of patients with ER+ breast tumors^[16]. Clinical studies have shown that ER+/PR+ tumors are more responsive to endocrine therapy than ER+/PR- tumors^[17]. Furthermore, down-regulation of PR correlates with high growth factor activity, indicating that loss of PR in ER positive breast tumors could serve as a predictor of endocrine therapy outcome^[16,17]. However, no biomarkers that predict resistance to endocrine therapy with certainty are available currently. Therefore most patients with ER positive breast cancers are treated with endocrine therapy, in adjuvant and/or metastatic setting. Tamoxifen is the treatment of choice in premenopausal patients. And aromatase inhibitors (*e.g.*, letrozole and anastrozole) have become the treatment of choice as first-line therapy in postmenopausal patients. On disease progression, second-line treatment options include other classes of AIs (steroidal or nonsteroidal) and the ER antagonists, fulvestrant and tamoxifen^[18]. But the effectiveness of endocrine therapy is limited by high rates of *de novo* or intrinsic resistance (existing before any treatment is given) and acquired resistance during treatment (resistance that develops during a given therapy after an initial period of response). One third of patients will have recurrent disease within 15 years after being treated with tamoxifen for 5 years^[11]. About 50% of patients with metastatic disease do not respond to initial endocrine treatment^[8]. Inevitably the vast majority of patients with ER-positive advanced breast cancer will become refractory to endocrine therapy.

A plethora of mechanisms have been proposed to explain resistance to endocrine therapy, including deregulation of various components of the ER pathway itself^[11,14,19], activation of escape pathways that provide tumors with alternative cell proliferative and survival stimuli^[20-24], alterations in cell cycle and apoptotic machinery^[3,25], modulation in epigenetics and microRNA profile^[1,4,6,26]. In this review, we summarize the key mechanisms that have been implicated in the development of endocrine resistance in breast cancer. We give an overview of the completed and ongoing clinical trials with novel agents targeting these alternative mechanisms, with the goal to overcome endocrine resistance in breast cancer.

LITERATURE SEARCH

PubMed was searched for articles in English published between January, 2000 to February, 2014 using the terms

“breast cancer”, “endocrine resistance”, as well as the individual terms of the molecular components under molecular mechanism listed in this Review. Reference lists from key articles were searched for additional material. Abstracts from the ASCO annual meetings and the San Antonio Breast Cancer Symposium were considered (2010-2012). ClinicalTrials.gov was searched for relevant trials. Articles were identified on the basis of the authors’ knowledge of the advances in endocrine resistant breast cancer research.

MOLECULAR MECHANISM OF ENDOCRINE RESISTANCE

De novo resistance in breast cancer is characterized by loss of ER (the ER α isoform) expression and ER gene mutations such as deletion and point mutation. Patients carrying inactive alleles of cytochrome P4502D6 (CYP2D6) deficiency cannot convert tamoxifen to its active metabolite, endoxifen, therefore are resistant to tamoxifen^[27]. By contrast, multiple mechanisms have been detected to account for the acquired resistance to endocrine therapies. Although it is beyond the focus of this review to summarize all of the known mechanism of endocrine resistance in breast cancer, we can focus on the molecular changes in some of the key pathways involved and their clinical implications (Figure 1).

DEREGULATION OF CLASSIC ESTROGEN SIGNALING

The classic function of ER is its nuclear function, also known as genomic activity, to regulate the expression of genes important for normal and cancer cell proliferation and survival^[3]. The nuclear estrogen receptors (ER α and ER β) have similar structure, consisting of a central DNA-binding domain flanked by two autonomous transcriptional activation domains. In classic estrogen signaling, ligand-bound ER activates gene expression-either through direct binding of dimeric ER to specific DNA response elements in complexes including co-activators, or function as a coregulator through protein-protein interactions with other transcription factors, such as activation protein 1 (Ap1), specificity protein 1 (Sp1) and nuclear factor (NF- κ B) to facilitate binding to serum response elements and activation of transcription^[28-30].

Mechanisms of endocrine resistance include the loss of ER α expression which occurs in 15%-20% of resistant breast cancers, ER α mutations which present in < 1% of ER-positive tumors, the expression of ER splicing variants, specifically the truncated variant ER α 36, and estrogen related receptors (ERR)^[11,14,31-33]. Deregulation of ER co-regulators has been implicated in endocrine resistance as well. For example, increased Ap1 and NF- κ B transcriptional activity are associated with endocrine resistance. Overexpression of nuclear

receptor co-activator 3 (nCOA3, also known as AIB1 or SRC3), detected in two-thirds of all breast cancers, has been implicated in clinical and experimental tamoxifen resistance^[3,21,34].

Post-translational modifications (phosphorylation, methylation and ubiquitination) of ER and its co-regulators are regulated to influence ER activity, interactions with other proteins including cytoplasmic signaling molecules^[21,35,36]. Aberrant regulations at this post-translational level contribute to endocrine resistance as well^[3].

ACTIVATION OF GROWTH FACTOR RECEPTOR PATHWAYS

The ER can also be activated by ligand independent fashion, as a consequence of signaling events downstream of membrane receptor tyrosine kinases (RTKs). RTKs are the intracellular portions of a class of growth factor receptors including HER2 (ERBB2), epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor (IGFR). The bidirectional crosstalk between the RTK signaling and ER pathways has been implicated in the development of resistance to endocrine therapy in preclinical studies. Many clinical trials have begun to test several attractive strategies, such as manipulation of growth factor signaling networks and the use of tyrosine kinase and multikinase inhibitors that may delay or even overcome the resistance of breast cancers to endocrine therapy.

HER2 pathway

HER2 (Human epidermal growth factor receptor 2/ERBB2) is a member of the HER receptor tyrosine kinase family, which plays an important role in promoting cell proliferation and malignant growth in breast cancer. Over-expression of HER2 occurs in approximately 30% of metastatic breast cancers (MBC) and is associated with aggressive disease course and poor outcome with reduced disease-free and overall survival rates. Both pre-clinical and clinical evidence suggested that HER2 over-expression confers resistance to anti-estrogen agents in ER positive tumors^[10]. Activation of the Her2 pathway, even without HER overexpression, confers tamoxifen resistance in ER positive cancer cells^[37]. Preclinical studies demonstrated that tamoxifen resistant cells have the ability to switch between HER2 and the ER pathway for cell growth and survival. Upregulation of HER2 signaling occurs in some tumors with disease progression during endocrine therapy. Recent studies show that HER2 gene expression is repressed by the PAX2-ER-tamoxifen complex in sensitive breast cancer cell lines; while in tamoxifen resistant cell lines, the ER coactivator AIB-1/SRC-3 competes with PAX2 for binding, leading to increased HER2 transcription^[38]. In addition, HER2 activation decreases ER level and increase ER phosphorylation, even in the absence of estrogen^[38-40]. HER2 signaling alters ER mediated transcription through disrupting the interac-

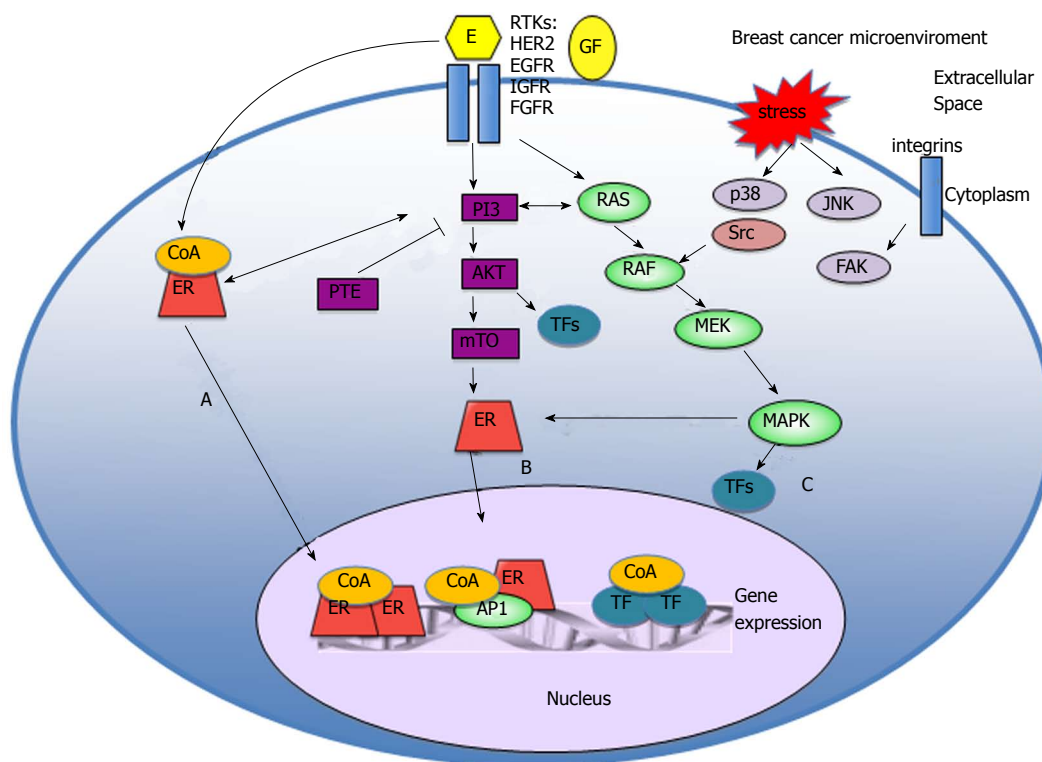


Figure 1 Estrogen receptor action at molecular level. A: Ligand dependent activation: in classic estrogen signaling, ligand-bound ER activates gene expression—either through direct binding of dimeric ER to specific DNA response elements in complexes including co-activators, or function as a coregulator through protein - protein interactions with other transcription factors to facilitate binding to serum response elements and activation of transcription; B: Ligand independent activation: the ER can also be activated by ligand independent fashion, as a consequence of signaling events downstream of membrane receptor tyrosine kinases (RTKs); C: Non-genomic mechanisms: signaling can be mediated through non-genomic mechanisms by ER that is localized at the cell membrane or in the cytoplasm. ER: Estrogen receptor; mTOR: Mammalian target of rapamycin; FGFR: Fibroblast growth factor receptor; IGF-1R: Insulin-like growth factor-1 receptor; EGFR: Epidermal growth factor receptor.

tion between ER and its coregulators (corepressors and coactivators). HER2 also activates downstream signaling pathways, such as the phosphoinositide 3-kinase (PI3K)/AKT pathway and mitogen activated kinase (MAPK) pathway, as discussed later^[3,15,19].

The interdependence of ER and HER2 pathways is highlighted by examples in which treatment with AIs or downregulation of ER with fulvestrant has inhibited the growth of HER2-positive tumors that had progressed with trastuzumab or lapatinib. In addition, HER2 inhibition with trastuzumab or lapatinib restores or upregulates ER levels or transcriptional activity in breast cancer cells^[24,41]. These data provide rationale for combined inhibition of ER and HER2 pathway, and clinical studies have demonstrated the benefit of targeting both the ER and HER2 in ER positive/HER2 positive breast cancer. In the phase III TAnDEM (Trastuzumab in Dual HER2 positive ER positive Metastatic Breast Cancer) trial, 207 postmenopausal women with HER2 positive ER positive MBC were randomized to anastrozole alone or anastrozole plus trastuzumab. The combination arm was clearly associated with a longer progression free survival (PFS) (4.8 mo *vs* 2.4 mo, $P = 0.0016$) and a higher clinical benefit rate (CBR) (42.7% *vs* 20.3%)^[42]. Similarly, in the randomized, double-blind phase III study of letrozole with or without lapatinib in MBC, PFS and clinical benefit

were superior in the combination arm compared with the AI-alone arm in 219 patients with ER positive/HER2 positive MBC^[43]. Both trials suggest that both HER2 and ER should be simultaneously targeted for maximal therapeutic efficacy.

EGFR pathway

Among the four HER family members (HER1-4), HER1 is better known as epidermal growth factor receptor (EGFR). Binding of EGF-related growth factors leads to receptor homo and/or heterodimerization (with HER2) and activation of downstream signaling cascades including PI3K/AKT and MARK pathways. In breast cancer, overexpression of EGFR and subsequently increased activity of MAPK and PI3K/AKT signaling pathways confer estrogen independency, resistance to endocrine therapy and poorer prognosis^[44-46]. For example, activation of ErbB3, EGFR and Erk is shown to be essential for growth of human breast cancer cell lines with acquired resistance to fulvestrant^[47]. In preclinical study, Gefitinib, a small molecule inhibitor of EGFR, effectively inhibited EGFR-HER2 heterodimerization, phosphorylation and downstream signaling in the tamoxifen resistant MCF-7 cell line^[48,49].

Lapatinib is a dual tyrosine kinase inhibitor blocking EGFR and HER2. In cell models of HER2 positive

breast cancer with acquired endocrine resistance, lapatinib restores hormone sensitivity^[50]. Johnston *et al.*^[51] reports in the randomized, double-blind phase III study, 1286 postmenopausal women with ER positive MBC, were randomized to receive letrozole with or without lapatinib. The benefit of combination therapy was observed in the ER positive, HER2 positive, but not in the ER positive, HER2 negative group. Letrozole plus lapatinib significantly increased PFS *vs* letrozole-placebo (8.2 mo *vs* 3.0 mo, HR = 0.71; 95%CI: 0.53-0.96; *P* = 0.019) in HER2 positive population^[46,51]. There was also a trend toward a prolonged PFS for the combination observed in patients who experienced relapse less than 6 mo since prior adjuvant tamoxifen discontinuation. These data suggest that there is benefit with the addition of an EGFR/HER2-targeted therapy to an AI in patients who experience relapse early during prior tamoxifen therapy which is consistent with preclinical models where EGFR activity is enhanced in association with endocrine resistance^[51].

Several selected EGFR inhibitors are being investigated as monotherapy or in combination with endocrine therapy in an attempt to overcome or prevent endocrine resistance. However, clinical trials that target EGFR in ER positive breast cancer have yielded mixed results. In the randomized placebo controlled phase II trial of tamoxifen with or without gefitinib, 290 patients were stratified into an endocrine naïve group who had not received endocrine therapy within one year prior to enrollment, and another group who had developed recurrence during or after AI therapy. PFS was not significantly prolonged in the endocrine naïve group (8.8 mo *vs* 10.9 mo, *P* = 0.31) or the group who had AI^[52]. Another small randomized placebo controlled phase II trial enrolled a total of 93 ER + metastatic breast cancer patients with or without prior endocrine therapy. In this study, combination of anastrozole with gefitinib showed a statistically significant increases in PFS compared to anastrozole plus placebo (14.7 mo *vs* 8.4 mo, HR = 0.55; 95%CI: 0.32-0.94). Similarly, subset analysis of PFS for patients who had received prior endocrine treatment compared with those who were endocrine therapy naïve showed a more pronounced benefit for patients that had not previously received endocrine therapy^[47]. These trials have suggested targeting EGFR could delay resistance to endocrine therapy in endocrine naïve patients.

Strategy of combined targeting the ER and EGFR was assessed in the neoadjuvant setting as well. Polychronis and colleague conducted the double-blind, placebo -controlled Phase II trial^[53]. 56 patients with ER and EGFR expressing breast cancer were randomized to receive gefitinib and placebo, or gefitinib plus anastrozole, for 4-6 wk prior to surgery. The combination arm showed a significant reduction in Ki67, which is the primary end point, than the monotherapy arm (5.6% difference, *P* = 0.0054). In contrast, Smith *et al.*^[54] reported a separate randomized phase II trial of neoadjuvant anastrozole alone or with gefitinib, in which 206 postmenopausal women with early stage ER positive breast

cancer were randomized to receive 16 wk of anastrozole monotherapy, 16 wk of anastrozole with 14 wk of gefitinib (preceded by two weeks of placebo) or 16 wk of gefitinib before surgery. There was no difference in proliferation index as measured by Ki67 for either gefitinib regimen when compared to anastrozole alone. Moreover, there was no difference in overall objective response (48% *vs* 61%, *P* = 0.08). The authors concluded that addition of gefitinib/EGFR inhibitor to neoadjuvant anastrozole did not improve clinical or biologic effect^[54]. The selection of EGFR overexpressing breast cancer cases in Polychronis *et al.*'s study might account for the difference in these trial results. One could postulate that the ideal setting for testing combination of endocrine therapy and EGFR inhibitors is in the patients with acquired resistance since it is associated with adaptive upregulation of growth factor receptor signaling. Further biomarker studies in patients who had prior endocrine therapy are clearly warranted to identify a phenotype that may predict relapse and subsequent benefit from combined endocrine therapy and EGFR inhibitors.

Mitogen activated kinase pathway

The mitogen activated kinase pathway (MAPK) pathway is stimulated by the RAF serine/threonine kinase, and signals to additional downstream cytoplasmic serine-threonine kinases that ultimately activate MAP kinases such as, ERKs, c-jun N-terminal kinases, and p38MAPKs with resultant downstream phosphorylation of transcription factors. As discussed earlier, the MAPK pathway is important in mediating HER2-and EGFR-induced endocrine resistance. In addition, studies show that ERK and p38 phosphorylate AIB1 and ER coactivators^[5,8]. Clinical trials targeting the MAPK pathway directly using MAPK inhibitors in combination with endocrine therapy are ongoing. Results on the randomized phase II trial, fulvestrant with or without AZD6244 (selumetinib, a MAPK Inhibitor) in advanced stage breast cancer progressing after aromatase inhibitor are awaited (NCT01160718).

The PI3K-AKT- mammalian target of rapamycin pathway

The PI3K-AKT (a serine/threonine kinase) pathway plays a central role in cell survival, proliferation and angiogenesis and is frequently deregulated in cancer^[45]. Phosphatidylinositol 3-kinase (PI3K) consists of a regulatory subunit (p85) and a catalytic subunit (p110). PI3K is activated by growth factor RTKs and G-protein-coupled receptors (GPCRs). PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5- trisphosphate (PIP3). In turn, PIP3 recruits several adaptor proteins such as phosphatidylinositol-dependent kinase 1 (PDK1) and AKT (a serine/threonine kinase), which when activated, drive cell proliferation and survival. Through dephosphorylation of PIP3 and PIP2 respectively, PTEN and INPP4B provide negative regulation of this pathway. AKT activates the mammalian target of rapamycin (mTOR) -containing complex 1 (mTORC1), which regulates protein synthe-

sis^[25]. Activating mutations or genetic amplification of PI3K catalytic subunit, amplification of downstream targets such as *Akt*, amplification of upstream receptors such as *erbB2/HER2* and loss of negative regulators such as *PTEN* have all been described in breast cancer^[55-57]. The Cancer Genome Atlas (TCGA) analysis confirms the high mutation frequency of *PIK3CA* in luminal/ER-positive breast cancer. *PIK3CA* somatic mutation is present in approximately 32% of luminal B subgroup, 49% of luminal A, 42% of HER2-enriched, and only 7% of basal-like breast cancer. Within the same pathway, *PTEN* mutation/loss and *INPP4B* loss were observed in more luminal B (24%, 16% each) than luminal A subtype (13% and 9% respectively)^[14,58]. The PI3K-AKT pathway is widely viewed as an important therapeutic target and PI3K pathway inhibitors are being studied in clinical trials.

Preclinical studies have associated PI3K pathway activation with *de novo* and acquired resistance to endocrine therapy. Increased phosphorylation of mTOR substrates and AKT is observed in estradiol deprived breast cancer cell lines. Oncogene overexpression that activate PI3K/AKT signaling (*e.g.*, HER2, type 1 insulin-like growth factor receptor (IGF1R), activated mutant AKT1) and RNAi-mediated knockdown of *PTEN* lead to resistance to tamoxifen, fulvestrant, and estrogen deprivation in ER-positive breast cancer cells. Studies using long-term estrogen-deprived (LTED) ER-positive breast cancer cell lines have shown that endocrine resistance develops concomitantly with amplification of PI3K/AKT/mTOR signaling^[59]. Similar changes have been observed with chronic exposure of MCF-7 cells and xenografts to fulvestrant^[23].

Moreover, inhibition of PI3K has reversed antiestrogen resistance in experimental models. For example, treatment with the PI3K/mTOR inhibitor BEZ235 or the mTOR inhibitor everolimus prevents the growth of LTED cell lines in the absence of estrogen^[60]. Everolimus in combination with tamoxifen had an additive anti-tumor effect in breast cancer cells *in vitro*^[61]. In another study, the combination of temsirolimus with an ER antagonist synergistically inhibited the growth of breast cancer cells *in vitro* and growth in a xenograft model of breast cancer (mTOR)^[62]. In a separate study, high levels of AKT activity conferred resistance to letrozole and fulvestrant through alteration of the cell cycle and apoptotic response in an *in vitro* breast cancer cell model^[63]. Treatment with everolimus plus either letrozole or fulvestrant restored responsiveness in the resistant cells and results in synergistic inhibition of the proliferation and induction of apoptosis^[60].

These preclinical studies indicated the promise of drugs targeting PI3K network (PI3K, AKT, mTOR) in ER positive breast cancer resistant to endocrine therapy. Table 1 summarizes the randomized trials in which inhibitors of PI3K pathway have been combined with endocrine therapy. Neoadjuvant treatment with letrozole and the mTOR inhibitor everolimus more effectively

reduced tumor cell proliferation and improved clinical response compared with letrozole alone in patients with early-stage ER-positive breast cancer^[64]. Two studies (BOLERO-2 and TAMRAD trials) have demonstrated superior benefit of mTOR inhibition in combination with endocrine therapy in advanced resistant ER positive breast cancers. In the phase III randomized BOLERO-2 trial, 724 patients with ER positive metastatic breast cancer (MBC) who had recurrence or progression while receiving previous therapy with a nonsteroidal aromatase inhibitor (either letrozole or anastrozole) were randomly assigned to everolimus and exemestane *vs* exemestane and placebo. The median PFS was significantly longer in the combination arm (10.6 mo *vs* 4.1 mo, HR 0.36; 95%CI: 0.27-0.47; $P < 0.001$, according to central assessment)^[65]. The combination of exemestane and everolimus has been approved for ER positive advanced breast cancer in United States and Europe based on the magnitude of these positive results. TAMRAD is a randomized phase II trial of tamoxifen with or without everolimus in patients with aromatase inhibitor (AI)-resistant metastatic breast cancer. Patients in the combination arm showed an improved clinical benefit rate (61% *vs* 42%), time to progression (8.6 mo *vs* 4.5 mo), and overall survival compared with patients receiving tamoxifen alone. Notably patients with acquired endocrine resistance (relapse > 6 mo after AI treatment) derived the greatest benefit from the combination compared with those with primary resistance (relapse during adjuvant AI or within 6 mo of AI treatment in the metastatic setting) with an improvement in the median PFS of 12.4 mo *vs* 1.5 mo, respectively^[66].

In contrast, Wolff *et al*^[67] examined letrozole with or without temsirolimus as first line therapy for patients with ER positive MBC who had no prior endocrine therapy for advanced disease in a randomized phase III trial. The study was terminated early due to lack of efficacy in the combination arm. Differences in results between the temsirolimus trial and the everolimus trials are likely attributable to different dosing schedules and pharmacokinetics, as well as different patient populations. It is possible that by selecting the more resistant cases, the TAMRAD and BOLERO-2 trials were enhanced with breast cancers that are likely to be driven by PI3K-mTOR signaling. Studies to identify predictive biomarker that could be used to select patients who would likely benefit from the combined mTOR and ER targeting approach are needed. In addition to mTOR inhibitors, drugs targeting other components of the PI3K pathway are in clinical development. Furthermore, isozyme-specific PI3K inhibitors have been developed in the hope of increasing therapeutic benefit while decreasing toxicity. Pan-PI3K inhibitors BKM120 and XL-147, dual PI3K/mTOR inhibitors BEZ235 and XL-765, and AKT inhibitor MK2206 have entered phase I, or phase I / II trials in combination of endocrine therapy.

Hedgehog signaling

The hedgehog (Hh) signaling pathway is highly conserved

Table 1 Clinical trials of targeted agents in endocrine resistant breast cancer

Agent	Class	Type of study	Study design	Patient population	Status/Results	Ref.
Targeting receptor tyrosine kinases signaling pathway						
PI3K/AKT/mTOR						
Everolimus	mTOR inhibitor	Phase III randomized	Exemestane +/- everolimus	ER+/HER2- LABC/MBC pts failed previous therapy with a nonsteroidal AI	PFS: 10.6 vs 4.1 mo, HR 0.36; $P < 0.001$, favoring combination arm	[76]
Everolimus	mTOR inhibitor	Phase II randomized	Tamoxifen +/- everolimus	ER+/HER2- MBC pts after previous therapy with AI	CBR: 61% vs 42%; TTP: 8.5 vs 4.5 mo, $P = 0.008$, favoring combination arm	[77]
Temsirolimus	mTOR inhibitor	Phase III randomized	Letrozole +/- temsirolimus	First line therapy for patients with ER positive MBC	No difference in CBR, terminated early	[78]
Everolimus	mTOR inhibitor	Phase II randomized	Letrozole +/- everolimus	Neoadjuvant therapy in ER + breast cancer	RR (by U/S): 58% vs 47%; $P = 0.035$, favoring combination arm	[75]
Sirolimus	mTOR inhibitor	Phase I / II	Tamoxifen +/- sirolimus	Pts with ER+ MBC	$N = 400$, TAM + SIR: 193; TAM alone: 207, ORR: TAM + SIR 40%; TAM alone 4%; Time to progression: TAM + SIR: 11 mo TAM alone: 3 mo	Bhattacharyya <i>et al</i> Eur.J.Cancer 47, Abstract 16LBA (2011)
Bkm120	Pan-PI3K inhibitor	Phase III randomized	Fulvestrant + BMK120	ER+/HER2- LABC/MBC Postmenopausal pts, AI Treated, Progressed on or After mtor Inhibitor		NCT01633060
Bkm120	Pan-PI3K inhibitor	Phase I b	Fulvestrant + BMK120	Postmenopausal pts with ER+ MBC	Ongoing, to determine the maximum tolerated dose of BKM120	NCT01339442
Bez235	Dual PI3K-mTOR inhibitor	Phase I b	Letrozole + BEZ235	Postmenopausal pts with ER+ MBC		NCT01248494
BMK120 or BEZ235	Pan-PI3K inhibitor	Phase I b	Letrozole +BMK120 or BEZ235	Postmenopausal pts with ER+ MBC		NCT01248494
XL147 or XL765	Pan-PI3K inhibitors/dual PI3K/mTOR inhibitor	Phase I / II	Letrozole +XL147 or XL765	ER+/HER2- MBC pts refractory to a previous AI therapy		NCT01082068
GDC-0941 or GDC-0980	dual PI3K/mTOR inhibitor	Phase II randomized	Fulvestrant +GDC-0941 or GDC-0980	Part I : ER+/HER2- postmenopausal LABC/MBC refractory to AI; part II : part I criteria pluspi3 camutation		NCT01437566
Gdc-0032	PI3K inhibitor	Phase I / II	GDC-0032 + fulvestrant	ER+/HER2- LABC/MBC Postmenopausal pts		NCT01296555
Byl719	PI3K- α inhibitor	Phase I	BYL719 + letrozole or exemestane	ER+/HER2- LABC/MBC pts		NCT01870505
Mk2206	AKT inhibitor	Phase I	Endocrine therapy + MK2206	Postmenopausal pts with ER+ MBC		NCT01344031
Mk2206	AKT inhibitor	Phase II	MK2206 monotherapy	LABC/LRBC/MBC withpi3ca mutation or AKT mutation or PTEN loss		NCT01277757
Azd5363	AKT inhibitor	Phase I / II	Paclitaxel +/- AZD5363	Parta: all MBC, partb: ER+ MBC, stratified by PIK3CA mutation		NCT01625286
Igf-1r Amg 479	IGF1R mAB	Phase II randomized	Addition of AMG 479 to either exemestane or fulvestrant	MBC or LABC pts who had progressed on prior endocrine therapy	No statistically significant difference in PFS (PFS: 3.9 vs 5.7 mo, favoring placebo arm, $P = 0.44$), OS or CBT between two arms	[87]
Bms-754807	dual IGF-1R/insulin receptor kinase inhibitor	Phase II randomized	BMS-754807 +/- letrozole	MBC or LA BC pts who had progressed on prior nonsteroidal AI		NCT01225172
Dalotuzumab (MK-0646)	IGF1R mAB	Phase I / II	MK-0646 and fulvestrant and dasatinib	ER+/HER2- MBC pts without prior therapy in metastatic setting		NCT00903006

Cixutumumab	IGF1R mAB	Phase I / II	Cixutumumab and temsirolimus	MBC or LA BC pts progressed on one to two chemotherapy	NCT00699491
Ridaforolimus (mk-8669) with dalotuzumab (mk-0646)	mTOR inhibitor and IGF-1R mAB		Ridaforolimus and dalotuzumab <i>vs</i> standard care	Er + bc	NCT01234857
Dovitinib (TKI258)	TKI inhibits FGFR1-3, VEGFR and PDGFR	Phase II Phase I / II	Dovitinib monotherapy, stratified by FGF amplification	4 groups of MBC pts: (group 1: FGFR1+, HR+), (group 2: FGFR1+, HR-) (group 3: FGFR1-, HR+), (group 4: FGFR1-, HR-)	Dovitinib has activity in breast cancers with amplified FGF pathway [94]
Dovitinib (TKI258)	TKI inhibits FGFR1-3, VEGFR and PDGFR		Dovitinib(TKI258) + AI	ER+/HER2- postmenopausal MBC resistant to AI with fgfr1 amplification status confirmed	NCT01484041
Dovitinib (TKI258)	TKI inhibits FGFR1-3, VEGFR and PDGFR	Phase II randomized	Fulvestrant +/- Dovitinib, stratified by FGF	Postmenopausal pts with HER2-/HR+ LA BC or MBC progressing within 12 mos of completion of adjuvant endocrine therapy or after ≤ 1 prior endocrine therapy in the advanced setting	NCT01528345
Azd4547		Phase II	amplification	HER2-MBC with fgfr1 amplification	NCT01795768
Azd4547		Phase II	Fulvestrant +/- AZD4547	ER+ postmenopausal LABC or MBC with fgfr1 polysomy or gene amplification resistant to endocrine treatment (Adjuvant or First-line Metastatic)	NCT01202591
Targeting cell cycle regulators					
Pd 0332991	CDK4/6 inhibitor	Phase I / II randomized	Letrozole +/- PD 0332991	First line therapy for postmenopausal pts with ER+/HER2- MBC	[99]
Pd-0332991 (palbociclib)	CDK4/6 inhibitor	Phase III randomized	Letrozole +/- PD 0332991	First line therapy for postmenopausal pts with ER+/HER2- MBC	NCT01740427
Lee011		Phase I b/ II	LEE011 + exemestane +/-everolimus	Postmenopausal pts with ER+/HER2- LABC/MBC	NCT01857193
Epigenetic therapy					
Vorinostat	HDAC inhibitor	Phase II	Vorinostat + tamoxifen	ER+ MBC progressed on previous endocrine therapy	N = 43; 34 evaluable, 7 (21%) PR; 4 (29%) SD; ORR 19%, CBR 40% [105]
Entinostat	HDAC inhibitor	phase II randomized	Exmestane +/- entinostat	MBC or LA BC pts who had progressed on prior nonsteroidal AI	N = 130; PFS: 4.3 <i>vs</i> 2.3 mo (HR 0.73, 95%CI: 0.50 to 1.07; P = 0.06); OS: 28.1 <i>vs</i> 19.8 mo (HR 0.59, CI, 0.36 to 0.97; P = .036), favoring combination [106]
Panobinostat	HDAC inhibitor	Phase I / II	Panobinostat + letrozole	MBC, triple negative phase II portion	NCT01105312
Vorinostat	HDAC inhibitor	phase II	Vorinostat + AI	ER + MBC pts who previously derived benefit from AI	NCT01153672
Vorinostat		phase II	Vorinostat/placebo + nab-paclitaxel + carboplatin (n = 62)	Primary operable breast cancer, triple-negative or high grade ER-positive, HER2-negative	Ongoing NCT00616967

MBC: Metastatic BC; LABC: Locally advanced BC; mAB: monoclonal antibody; ORR: Objective response rate; CBR: Clinical benefit rate response or stable disease >24 wk; PR: Partial response; SD: Stable disease; TKI: Tyrosine Kinase Inhibitor; PI3K/AKT/mTOR: PI3K-AKT- mammalian target of rapamycin (mTOR) pathway; IGF1R: Insulin-like growth factor-1 receptor pathway; FGF: Fibroblast growth factor. signaling.

and plays a critical role in embryonic development. The Hh pathway has been increasingly recognized as playing

a crucial role in carcinogenesis in the last decade. Three mammalian Hh ligands have been identified in humans, as denoted by the prefixes Sonic, Indian, and Desert (SHH, IHH, and DHH). They activate the Hh signaling pathway by binding to the cell surface receptor Patched (PTCH), which otherwise represses the activity of the transmembrane receptor like protein Smoothed (SMO). Release of SMO from PTCH-mediated repression subsequently leads to the modulation of GLI (glioma-associated oncogene homolog) transcription factors. There are three mammalian GLI proteins, GLI1, GLI2 and GLI3. GLI1 is a transcriptional activator; GLI2 can either activate or repress gene expression; GLI3 acts as a transcriptional repressor. Aberrant activation of the Hh pathway has been reported in several malignancies including breast cancer^[68,69].

Traditionally, four major mechanisms have been proposed account for aberrant activation of the Hh pathway: (1) Hh ligand-independent mechanism - Loss of function mutations in PTCH or gain of function mutations in SMO lead to constitutive activation of this pathway; (2) Autocrine signaling- tumor cells produce Hh ligand to activate the Hh signaling; (3) Paracrine signaling - Hh ligand produced by tumor cell stimulates stromal and endothelial cells that produce growth factors supporting tumor growth and survival; and (4) Reverse paracrine signaling-Hh ligand produced by stromal cells support tumor growth and survival. Upon the pathway activation, the GLI transcription factors activate or inhibit transcription by binding to their responsive genes and interacting with the transcriptional complex. A ligand-dependent autocrine model of activating the Hh signaling has been described in breast cancer^[69,70].

We recently show noncanonical Hh signaling as an alternative growth promoting mechanism that is activated in tamoxifen-resistant breast tumors. Importantly PI3K/AKT pathway plays a critical role in regulating Hh signaling by protecting key components of this pathway from proteasomal degradation. We showed that activation of Hh signaling correlated inversely with disease-free and overall survival in a cohort of 315 patients with breast cancer with poor disease outcome. Furthermore, we observed that among ER positive, node-positive patients, Hh activation in the primary tumors was an independent prognostic factor for worse disease-free survival. Add treatment of tamoxifen-resistant xenografts with anti-Hh compound GDC-0449 blocked tumor growth in mice. These promising preclinical results describe a signaling event linking PI3K/AKT pathway with Hh signaling that promotes endocrine resistance^[71]. Targeting Hh pathway alone or in combination with PI3K/AKT pathway could therefore be a novel therapeutic option in treating endocrine resistant breast cancer. We are currently planning a phase I / II clinical trial using GDC-0449 (vismodegib), an oral compound approved for the management advanced basal cell carcinomas in patients with ER positive MBC that are resistant to endocrine therapy. Interestingly, Hh signalling has been shown to condition the bone mi-

croenvironment for osteolytic metastasis of breast cancer^[45], therefore Hedgehog inhibitors are candidate drugs for the treatment of patients with bone metastases which is the most common site of metastasis in ER positive breast cancer.

Insulin-like growth factor-1 receptor pathway

Studies have shown that ligand activation of Insulin-like growth factor-1 receptor (IGF-1R) and its downstream pathways stimulate tumor growth by inhibition of apoptosis and promotion of transformation, metastasis and angiogenesis^[72]. IGF-1R is expressed in 90% to 95% of breast cancer and is often co-expressed with ER^[73]. The crosstalk between IGF-1R and ER pathway is critical for the development of IGF-1R -mediated endocrine resistance in breast cancer. For example, estrogen activates IGF1R pathway through genomic and nongenomic mechanism. IGF-1R plays a direct role in ER phosphorylation. In addition, activation of IGF-1R signaling is associated with loss of PR expression, which itself is associated with high proliferative ER positive breast cancer^[74]. IGF1R overexpression also renders resistance to tamoxifen and fulvestrant through activation of MAPK and PI3K pathway.

Multiple agents interrupting the IGF-1 signaling pathway are developed and tested in clinical trials. AMG 479, a humanized monoclonal antibody antagonist of IGF1R, is tested with exemestane or fulvestrant in postmenopausal women with ER positive locally advanced or metastatic breast cancer who had disease progression on prior endocrine therapy in a randomized phase II trial. No statistically significant difference in PFS (PFS: 3.9 mo *vs* 5.7 mo, favoring placebo arm, $P = 0.44$), OS or CBT between two arms in this study^[75]. Ongoing trails with IGF-1R inhibitors are listed in Table 1. Correlative studies of these trials will be critical to determine whether there is a benefit adding IGF-1R inhibition to anti-estrogen therapy in patient cases with aggressive features, such as increased proliferation.

Fibroblast growth factor signaling

Fibroblast growth factor receptor (FGFR) signaling system includes at least 18 FGF ligands and four transmembrane tyrosine kinase FGF receptors, and it is involved in cancer cell proliferation, migration, angiogenesis, and survival^[76]. Multiple studies indicate that deregulated FGFRs can function as driving oncogenes stimulating tumorigenesis in a variety of human malignancies in addition to its role as an escape mechanism of anti-VEGF (vascular endothelial growth factor) therapies^[76,77]. A variety of FGFR pathway alterations have been identified in cancer and include activating mutations; chromosomal translocations resulting in expression of FGFR-fusion proteins with constitutive FGFR kinase activity; aberrant splicing of *FGFR* and isoform switching which substantially alter ligand specificity; gene amplifications or receptor overexpression through post-transcriptional regulation. Subsequently, aberrant activation of downstream path-

ways results in mitogenic and antiapoptotic responses in cells^[78,79].

FGFR family members are frequently overexpressed in breast cancer^[28]. *FGFR1* is the most commonly amplified genes following *erb2/HER2* in breast cancer, present in about in 8%-15% of all breast cancer^[14,76]. Large series have shown that *FGFR1* amplification is associated with high proliferation as assessed by Ki-67 immunostaining, drives resistance to endocrine therapy and is an independent predictive factor of poor prognosis^[22].

Preclinical models of breast cancer cells with amplification of *FGFR1* or *FGFR2* have demonstrated sensitivity to inhibition of FGFR^[80]. Several antibodies and small molecule inhibitors of FGFR are currently in early-phase clinical trials. Dovitinib (TKI258) is a first generation oral tyrosine kinase inhibitor (TKI) which inhibits *FGFR1-3*, *VEGFR* and platelet-derived growth factor receptor (*PDGFR*). Dovitinib inhibits proliferation in *FGFR1*- and *FGFR2*- amplified, but not *FGFR*-normal, breast cancer cell lines. Dovitinib monotherapy was evaluated in the phase II trial selecting patients on the basis of hormone receptor (HR) status and *FGFR1* amplification status. The mean reduction in target lesions was 21.1% in patients with FGF pathway-amplified breast cancer based on qPCR assay, compared with a 12.0% increase in target lesions in patients who did not present with FGF pathway-amplified breast cancer. Therefore, preliminary results suggest Dovitinib has antitumor activity in advanced breast cancer with FGF pathway alterations and warrants further investigation^[81].

CELL CYCLE SIGNALING AND APOPTOSIS

Experimental model data and clinical correlations indicate anti-estrogen treatment leads to a G₁ phase-specific cell cycle arrest and reduction in growth rate. Several molecular consequences that result in apoptosis have been documented. Aberrant regulation of positive and negative regulators of the cell cycle has been shown to interrupt and inhibit the antiproliferative effects of endocrine therapy, leading to treatment resistance^[3]. For example, overexpression of the positive regulators *MYC*, cyclins *E1* and *D1* cause endocrine resistance either by activating cyclin-dependent kinases critical for G₁ phase or by relieving the inhibitory effects of the negative cell cycle regulators *p21* and *p27*^[3,74]. Importantly, expression and activity of these negative cell cycle regulators are down-regulated by multiple growth factor receptors and their downstream signaling pathways by modulating specific transcription factors, microRNAs, or by interfering protein phosphorylation. Moreover, increased expression of anti-apoptotic molecules such as *BCI-2* and *BCI-X1* and decreased expression of pro-apoptotic molecules such as *BAK*, *BiK* and *caspase 9* lead to endocrine resistance as well^[82]. Of note, activation of growth factor receptor signaling *via* the *PI3K/AKT* pathway is critical modulators of many apoptotic/survival molecules^[83]. Cyclin *D1* is a

well-studied ER target gene that is required for estrogen-induced cell proliferation. Cyclin *D1* binds to and activates cell cycle-dependent protein kinases four and six (*CDK4/6*) essential for mediating RB-induced cell cycle progression at the G₁/S checkpoint^[53,74]. Cyclin *D1* amplification and overexpression was a common oncogenic event in breast cancer and preferentially occurred within luminal tumors, and more specifically within luminal B subtype. In the Cancer Genome Atlas (TCGA) network studies, Cyclin *D1* is amplified in 58% of luminal B breast cancers with *CDK4* gain in 25% of this subtype. In comparison, only 29% of luminal A tumors has Cyclin *D1* amplification with 14% has *CDK4* gain^[14]. Furthermore, Wang *et al*^[84] report that the alternatively spliced message, cyclin *D1b*, is aberrantly regulated in response to therapeutic challenge and promotes resistance to estrogen antagonists. Recently, Thangavel *et al*^[85] noted that a unique gene signature indicative of RB protein loss of function could identify luminal B breast cancers most likely to be resistant to endocrine therapies. Therefore targeting cyclin *D1* and its downstream mediators of ER action *CDK4/6* may provide a viable strategy to treat endocrine resistant breast cancers.

A phase I / II clinical trial testing the efficacy of letrozole with or without PD-0332991 (an oral *CDK4/6* inhibitor) was conducted as first-line treatment of ER-positive advanced breast cancer (NCT00721409). This trial excluded patients who have previously been treated for advanced breast cancer. Thus the patient population is not determined to be endocrine resistant. The preliminary results were very impressive and showed significant prolongation of median PFS with the combination when compared to letrozole alone (26.2 mo *vs* 7.5 mo; HR = 0.32, 95%CI: 0.19-0.56, *P* < 0.001)^[86]. The result of the randomized, multicenter, double-blind phase III study of palbociclib (PD-0332991), plus letrozole *vs* placebo plus letrozole for postmenopausal women with ER positive, HER2 negative MBC who have not received any prior systemic treatment for advanced disease is awaited (NCT01740427)^[87]. Trials using other *CDK* inhibitors (Novartis) are also underway.

EPIGENETICS AND ENDOCRINE RESISTANCE

Epigenetics is defined as reversible changes in gene expression without change in the DNA sequence. DNA methylation is mediated by the action of DNA methyltransferases (*DNMTs*). *DNMTs* directly interact with histone deacetylases (*HDACs*) and the methyl-CpG-binding domain (*MBD*) family of proteins at the promoter regions to form a repressive transcription complex. DNA methylation, histone modification, and nucleosome remodeling are the major epigenetic changes that are dysregulated in breast cancer. Several genes involved in proliferation, anti-apoptosis, invasion, and metastasis have been shown to undergo epigenetic changes in breast cancer^[88,89].

There is increasing evidence that epigenetic modification plays a potential role in the development of endocrine resistance in breast cancer. The epigenetic regulation of ER is mediated through the recruitment of multi-molecular complexes containing HDAC1, DNMT1 and other co-repressors to the promoter region. Methylation of the gene encoding ER- α is one of the mechanisms of loss of ER expression in ER negative breast cancer cell. The epigenetic silencing of ER target genes is crucial to the development of ER independent growth and endocrine treatment resistance. A number of preclinical studies have shown that epigenetic therapy can impact expression of ER. For example, inhibition of DNMTs in ER negative breast cancer cells leads to induction of ER expression^[90,91]. HDAC inhibitors can restore ER expression, either alone *via* chromatin remodeling or in combination with DNMT inhibitors^[89]. The TCGA study highlights the finding that breast cancer molecular subtypes harbor specific patterns of epigenetic hardwiring and further demonstrates luminal B is a distinct subtype from luminal A not only based on the mRNA-based assay but also at the methylation and protein levels^[14]. Five DNA methylation groups were identified from 802 patient samples. Interestingly, the hypermethylated group 3 was significantly related to Luminal B subtype. Comparison between DNA methylation status and mRNA expression profile of group 3 with other groups led to identification of over 4000 differentially methylated genes and almost 2000 differentially expressed genes^[14]. Collectively, these data provide basis for the biological rationale for combining endocrine therapy with epigenetic-targeted therapies.

A phase II study of vorinostat, a HDAC inhibitor, in combination with tamoxifen was conducted in MBC patients who had progressed on previous lines of hormone therapy^[92]. The overall response rate was 19% and CBR was 40% (defined as Complete Response, Partial Response or Stable Disease of > 6 mo in duration) in 43 patients treated. The results from the randomized double blind phase II study of exemestane with or without entinostat, a benzamide HDAC inhibitor, are promising for reversal of AI endocrine therapy resistance. 130 postmenopausal women with locally recurrent or metastatic ER-positive breast cancer progressing on treatment with a nonsteroidal AI were enrolled. In this study, PFS was 4.3 mo *vs* 2.3 mo (HR 0.73, 95%CI: 0.50-1.07, $P = 0.055$) and OS was 28.1 mo *vs* 19.8 mo (HR = 0.59, 95%CI: 0.36-0.97) for the group receiving combination therapy *vs*. exemestane alone^[93]. Trials combining letrozole and panobinostat, vorinostat and AI therapy in metastatic breast cancer, vorinostat and tamoxifen in early stage breast cancer, are ongoing. Based on the higher frequency of methylation observed in Luminal B tumors, it is possible that luminal B breast cancers may represent a better target for epigenetic therapy than other subtypes.

MICRO RNA

Micro RNA (miRNAs) is a class of small noncoding,

single-stranded, highly conserved RNAs (19-25 nucleotides) involved in essentially all aspects of physiological and pathological cellular processes, such as development, proliferation, differentiation and apoptosis. MiRNA can either cleave mature mRNA molecules or inhibit their translation through base-pairing within the 3'-UTR of protein coding genes. Research over the past decade has demonstrated that about one third of human genes appear to be targeted by miRNAs and each miRNA is thought to regulate multiple genes. Interestingly, specific miRNA signatures have been associated with different molecular subtypes of breast cancer. In the Cancer Genome Atlas network analysis, 7 breast cancer subtypes were identified on the basis of MiRNAs expression and correlated with molecular subgroups^[14]. We have explored the potential role of specific miRNAs in endocrine resistance, especially resistance to tamoxifen, in breast cancer. Studies from our and other groups showed miR-221, miR-222 and miR-181b are up-regulated, whereas miR-21, miR-342 and miRNA-489 are downregulated in the tamoxifen-resistant cells. Multiple mechanisms of these miRNAs in conferring resistance to tamoxifen have been published. Mir-221 and -222 target the cell cycle inhibitor, p27/Kip1 through posttranslational modification and sequestration of p27 protein, or through miRNA-mediated suppression. Mir-221 and -222 overexpression is known to suppress ER α expression at protein level which leads to tamoxifen resistance in ER positive breast cancer^[19]. We recently reported that TIMP3, a tissue metalloproteinase inhibitor, is down-regulated by miR-221, -222 and -181b. We showed miRNA-mediated regulation of TIMP3 level and inhibition of metalloproteases contribute to tamoxifen resistance in cell culture models, mouse xenograft models, as well as in primary breast tumors. Direct injection of antago miRNA-221/222 to tamoxifen resistant xenografts in mice caused decrease in miRNA-221/222 level and restoration sensitivity to tamoxifen^[94]. Other groups subsequently reported up-regulation of miR-221 and -222 is implicated in resistance to fulvestrant as well^[52].

Investigation during the last decade demonstrate emerging regulatory role of miRNAs in endocrine resistant breast cancer. Future studies evaluating miRNAs as prognostic and predictive markers, as well as novel therapeutic targets to overcome resistance are warranted.

CONCLUSION

Recent progress in the field of endocrine therapy has produced a significant number of active compounds. Patients with ER-positive advanced breast cancer are treated with different endocrine agents serially at tumor progression, often resulting in long periods of disease control with no significant toxicity. Inevitably, however, vast majority of patients will become refractory to endocrine therapy. Therefore resistance to endocrine therapy continues to be a subject of great importance. In this review, we have summarized the complex genomic and epigen-

etic regulatory pathways involved in endocrine resistance. A combination of ER-targeted and HER2- targeted therapies is our current standard-of-care therapy in ER positive, HER2 positive breast cancer. Early results from clinical trials suggest that subsets of patients may benefit from a combination of inhibitor targeting certain growth factor pathway with endocrine therapy. The combination of exemestane and mTOR inhibitor everolimus has been approved for ER positive advanced breast cancer in USA and Europe based on the magnitude of positive results in two randomized phase III trials. The use of epigenetic therapy or miRNA/antimiRNA-based therapy with existing endocrine therapy in breast cancer is a topic of active interest.

Many challenges still remain as we try to identify the subsets of patients most likely to benefit from these novel targeted agents. Efforts should be directed at defining biological markers that could predict the efficacy of a specific agent. The use of genome-wide approaches in detecting gene alterations that drive resistance to endocrine therapy will hopefully promote personalized cancer medicine in management of endocrine resistance breast cancer. Clearly, future clinical trials with prospective patient selection based on predictive biomarkers are needed.

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