CLINICAL INVESTIGATION

Endocrine resistance: mechanisms and therapeutic targets

Endocrine therapy remains the cornerstone in the effective management of estrogen receptor-positive breast cancers. Since the introduction of tamoxifen, the first therapy to target estrogen receptor, several agents have been approved to block the estrogen-mediated signaling in breast cancer management. However, successful clinical outcomes are hindered by the alternate survival mechanisms either innately active or developed by the cancer cell over time. Identifying these signaling pathways at the outset or over the treatment period, and developing targeted therapies to inhibit these pathways, would be crucial to improve clinical outcomes. This article reviews the landscape of the resistant pathways identified so far and the translational work that aims to target these pathways to enhance the effectiveness of endocrine therapy.

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Recent advances in molecular profiling have not only helped us to better understand the biology of cancers, but have also helped to identify drug targets of therapeutic benefit. However, not all tumors harboring a particular target or mutation respond to the specific drug, and even the tumors that respond initially cease to respond at some point, underscoring the importance of resistance pathways. Breast cancers are the second most common type of cancers worldwide with one in eight women having a lifetime risk of developing breast cancer. Of these, approximately 70% express estrogen receptors (ERs) and are categorized as endocrine-positive breast cancers [1]. Endocrine therapies targeting the proliferative effect of estrogen, mediated through ERs are the cornerstone for treating hormone receptor positive breast cancers. Hormonal agents such as tamoxifen and aromatase inhibitors (AIs; e.g., letrozole and exemestane) play a pivotal role in the treatment of these cancers [2]. However, even the tumors that respond initially eventually develop resistance to these agents through molecular mechanisms that are not yet completely discerned. Understanding the molecular mechanisms that underlie endocrine resistance is essential for the discovery of rational combinatorial therapies. This article discusses the mechanisms and the putative approaches to overcome resistance to endocrine therapy.

ER-mediated signaling

The role of estrogen in the pathogenesis of breast cancers is well established. Consequently, therapies to block signaling through ERs or reduce the production of estrogen form an integral part of the systemic treatment of ER-positive breast cancers.

ERs belongs to the nuclear receptor family that includes vitamin A and D, as well as thyroid hormones [3]. When bound to their ligand, the ERs act as transcriptional factors for specific target genes. There are two different forms of ER, ER-α and ER-β, encoded by distinct genes located on chromosomes 6 and 14, respectively [4]. The gene encoding the progesterone receptor (PR) is ER-related

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and its transcription depends on ER activation. Tumors that express both ERs and PRs are considered more endocrine responsive and, consequently, the absence of PRs has been associated with poor outcomes in breast cancer patients [5]. ER-α and ER-β have tissue-specific expressions with some overlap. ER-α is mainly expressed in the mammary glands, ovaries (thecal and interstitial cells), uterus, liver, kidneys and adrenal glands, whereas ER-β is more highly expressed in prostate, bone, lungs and granulosa cells of the ovaries [6,7]. Both ER-α and ER-β share a high degree of homology in their DNA and ligand-binding domains [8], whereas, the N-terminal A/B domain and the C-terminal F domain are variable. In comparison with ER-α, ER-β also exhibits a weak AF1 activity with differential response to ligands, resulting in differences in specific actions of estrogens across different target tissues [7]. ER-α is thought to play a crucial role in breast cancer initiation and progression, whereas the role of ER-β is poorly understood [9]. An increase in ER-α relative to ER-β has been observed in breast cancers, suggesting that ER-β is likely to be associated with carcinogenesis and ER-β has a protective effect [10,11]. Typically, ER positivity in breast cancers refers to immunohistochemical evaluation for ER-α.

ER acts through genomic and non-genomic mechanisms that are both complementary as well as synergistic [7]. Genomic- or nuclear-initiated steroid signaling mechanisms of signaling can be classified as classical or nonclassical. In the classical pathway, ERs remain in the cytoplasm as inactive monomers bound to heat-shock proteins. Binding of estrogen to ER induces a conformational change leading to dissociation of heat-shock proteins and dimerization of the receptor. The ER dimer then binds to the consensus sequences known as estrogen-response elements, located in the promoter region of genes regulated by estrogen, resulting in cell proliferation and survival. While binding of estrogen to ER recruits co-activators activating transcription, binding of antagonists such as tamoxifen would lead to recruitment of co-repressors, thus inhibiting gene transcription. In the nonclassical pathway, ER can also influence the transcription of genes lacking estrogen-response elements and is involved in the transcription of genes such as CCND1 and IGFR1. This pathway does not require direct binding of ER to DNA sequences; instead, transcription is mediated through protein–protein interactions with members of the Fos/Jun family.

On the other hand, signaling through the membrane-associated ER is rapid and independent of gene transcription at the outset [7]. This ligand-dependent pathway is called membrane-initiated steroid signaling. At the membrane, ER associates with caveolin rafts and forms dimers upon estrogen binding and interacts with adaptor proteins such as Src and G-proteins. This results in activation of growth factor receptors (GFR) such as EGF receptor (EGFR), and IGF receptor (IGFR), which activates kinases belonging to the PI3K/AKT/mTOR and the MAPK pathway [7]. In turn, these kinases can phosphorylate ER and its coregulators, resulting in the activation of genomic signaling pathways [12]. Nongenomic activity is highly regulated by coregulatory proteins, and is influenced by signal transduction pathways operating in tumor milieu or specific tissue [7]. In tissues such as bone and endothelial cells, estrogen signaling is predominantly nongenomic [13]. Important coregulators include proline–glutamic acid, PELP1 or MNAR, and the metastasis-associated proteins [14,15]. PELP1 is overexpressed in breast cancers and enhances both genomic and non-genomic activity, whereas MTA potentiates nongenomic actions of ER while inhibiting genomic pathways. The ER and GFR pathways have bidirectional signaling suggesting that the GFR pathway could mediate endocrine resistance.

Typically, ER-positive breast cancers with low grade and low proliferation rate, as well as low prevalence of HER2 (ErbB2) amplification with frequent metastasis to bone and soft tissues [16]. However, molecular profiling has enabled us to recognize the heterogeneity within the ER-positive breast cancers [17]. Two distinct molecular subtypes, luminal A and B, have been characterized. The luminal A forms of ER-positive breast cancers are characterized by lower proliferation rates, less aggressive tumor behavior with high ER expression and thus increased responsiveness to endocrine therapy. On the other hand, luminal B forms of ER-positive breast cancers are typically associated with increased aggressiveness, higher proliferation rates and relatively lower expression of ER [18].

Hormonal agents

Endocrine therapy for patients with ER-positive breast cancers represents the earliest and remains the most effective form of targeted therapy [1]. However, resistance – both intrinsic and acquired – occurs in a significant proportion of patients, limiting the efficacy of endocrine treatments. Three classes of hormonal agents have been successfully used in the adjuvant and metastatic setting to treat ER-positive breast cancers. They are selective ER modulators (SERMs), aromatase inhibitors (AI) and selective ER downregulators (SERDs) (Table 1).

SERMs, such as tamoxifen and toremifene, are a class of compounds with mixed agonist/antagonist activity that may either stimulate or inhibit ER function depending on the tissue [19]. They competitively inhibit binding of estrogen to ER and affect both the

genomic and non-genomic pathways. Tamoxifen, the most widely studied and used SERM, binds to ER in the same manner as estrogen and induces its dimerization. However, it recruits corepressors and inhibits AF-2-dependent interaction with coactivators, thus blocking gene transcription [20]. The agonistic or antagonistic effect of tamoxifen in different tissues is determined based on the availability of co-activators or corepressors and relative expression of AF1- or AF2-dependent genes [7]. In addition to the genomic effects, tamoxifen also influences the non-genomic ER signaling in tumor cells expressing abundant EGFR, HER2 or PELP1, leading to cell growth and resistance through the membrane-associated ER pathway. On the other hand, estrogen-deprivation therapy with AI can inhibit both nuclear-initiated and membrane-initiated steroid signaling of ER, and thus might be more effective in HER2-positive breast tumors. This has been corroborated in neo-adjuvant clinical trials that compared AI with tamoxifen in HER2-positive patients [21,22]. Tamoxifen is used both in the adjuvant as well as in the metastatic setting in both pre- and post-menopausal women.

AIs inhibit estrogen synthesis and represent an alternative therapeutic strategy to ER antagonism. Two classes of AIs are currently used clinically: steroidal (e.g., exemestane), which binds aromatase irreversibly, and nonsteroidal (e.g., anastrazole and letrozole) that block the enzyme reversibly [23]. Besides the ovaries, estrogen is also produced in adipose tissues by the cytochrome P450 aromatase enzyme. This enzyme converts androstenedione and testosterone to estrone and estradiol, respectively, in the adrenal gland, adipose tissue and breast. Thus, AIs inhibit the activity of this enzyme and decrease estrogen levels. Evidently, this effect is more pertinent in postmenopausal women as estrogen is produced by aromatization of androgens peripherally. Therefore, they are the anti-estrogens of choice for adjuvant and palliative use in postmenopausal women. Additionally, in premenopausal women, ovarian suppression can be achieved using GnRH agonists, such as goserelin, which inhibits the release of the pituitary hormones FSH and LH and consequently blocks estrogen production by the ovaries. These can be used in combination with AIs to inhibit estrogen activity.

SERDs (e.g., fulvestrant), similarly to SERMs, compete with estrogen for binding with ERs. However, these agents also block receptor dimerization and induce degradation of receptor proteins. SERDs completely inhibit ER-mediated gene transcription, inactivating both AF1 and AF2, resulting in complete inhibition of estrogen activity [7]. Fulvestrant is currently US FDA approved for the treatment of ER-positive metastatic breast cancer patients with progressive disease following failure of previous anti-estrogen therapies.

Table 1. Anti-estrogen therapy used in estrogen receptor-positive breast cancers.	
Drug class	Agents
Selective estrogen receptor modulators	Tamoxifen, raloxifene, torimefene
Aromatase inhibitors	Nonsteroidal – letrozole, anastrazole Steroidal – exemestane
Selective estrogen receptor down regulators	Fulvestrant

Mechanisms of resistance to endocrine therapy

While anti-estrogen therapies have long been the cornerstone of treatment in patients with ER-positive breast cancers, resistance to therapy remains a challenge, evading cure for several patients. Observed resistance to endocrine therapy can be primary (*de novo*) or secondary (acquired) in nature and this can be specific to a particular agent or class of drugs. Multiple mechanisms responsible for endocrine resistance have been proposed. They include deregulation in ER or various components of the ER pathway, alterations in cell cycle and survival signaling molecules, alterations in growth factor signaling and the activation of alternate survival pathways.

■ Changes in ER & coregulators

Evidently, expression of ER is the single major determinant of response to therapy, and tumors that lack ER show innate resistance to endocrine treatment. Patients carrying inactive alleles of cytochrome P450 2D6, observed in 8% of Caucasian women, show intrinsic resistance to tamoxifen, as they are unable to convert tamoxifen to the active form endoxifen [24]. While mutation in ER-α is likely to affect response to endocrine therapy, these are rarely noted in human breast cancers, thus their contribution is not very significant [25]. Loss of ER-α expression, post-translational modifications, expression of truncated isoforms of ER-α and ER-β and deregulation of ER coactivators are likely to contribute to endocrine resistance. Loss of ER-α expression occurs in 15–20% of endocrine-resistant cancers and less than 1% of tumors have mutations in ER-α [26]. Expression of a truncated form of ER-α, ERa36, has been reported to mediate tamoxifen resistance [27]. Chromatin modification, mediated by DNA methylation and histone deacetylation, can lead to silencing of ER-α [28]. Multimolecular complexes involving HDAC and DNMT have been identified as important factors in the regulation of *ESR1* gene expression [29].

Recently, Pan *et al.* have shown that the ubiquitin-binding CUEDC2 binds to ER-α and causes degradation [30]. They reported that overexpression of CUEDC2 led to tamoxifen resistance and reduced survival of

ER-positive patients. Interestingly, CUEDC2-mediated resistance was rescued by coexpression of ER-α, suggesting that its effects are predominately mediated through ER-α. LMTK3 has also recently been identified as a negative regulator of ER-α. High LMTK3 levels at baseline were predictive for endocrine resistance and *LMTK3* gene amplification was associated with relapse while receiving tamoxifen, supporting the role of LMTK3 in mediating resistance [31].

ER-α levels are under complex regulation by multiple transcription factors. Increased Ap1 and NF-κB transcriptional activity have been associated with endocrine resistance [32]. Increased expression of nuclear receptor coactivator 3 (NCOA3) is also associated with reduced responsiveness to tamoxifen and confers resistance [33].

■ Role of PI3K signaling

The PI3K AKT/mTOR is a central regulatory pathway involved in cell proliferation, growth and survival. Deregulation of this pathway has been associated with the development of resistance to endocrine therapy [34]. Class IA PI3Ks are activated by receptor tyrosine kinases and serve as a cardinal pathway for transduction of molecular signals. PI3K/AKT signaling pathway regulates the G1/S transition of the cell cycle by activating mTOR [7]. Phosphotyrosine residues of receptor tyrosine kinases interact with the p85 regulatory subunit and activate PI3K to convert PIP2 to PIP3. PIP3 recruits AKT to the plasma membrane and activates it. Activated AKT phosphorylates intracellular proteins, including mTOR, which subsequently phosphorylates S6 kinase 1 and 4EBP1 that are crucial in regulating cell-cycle progression. Increased AKT activity is observed in 20–55% of ER-positive breast cancers and is associated with relapse and death [35,36]. PTEN, on the other hand, negatively regulates PI3K by dephosphorylating PIP3 [37]. PIK3CA mutation is the most common genetic abnormality in ER-positive breast cancer patients; however its prognostic implication is uncertain [38]. Gene expression studies have shown that PIK3CA mutations associated with low mTORC1 signaling was associated with good clinical outcomes with hormonal therapy in ER-positive, HER2-negative tumors [39]. Activation of the PI3K signaling is associated with the luminal B subtype of breast cancer and correlates with endocrine resistance [37]. PI3K pathway activation leads to estrogen-independent activation of ER-α, leading to upregulation of estrogen-regulated genes such as *Bcl-2* and inhibition of mTOR activity restored response to tamoxifen in breast cancer cell lines with aberrant AKT activity [40,41].

A recent study has identified the cross talk between PI3K/AKT and Hedgehog (Hh) pathway [42]. PI3K/AKT pathway activated Hh-signaling molecules SMO and GLI1 in tamoxifen-resistant breast cancer

cell lines, and treatment with PI3K inhibitors abrogated this effect. Furthermore, treatment with GDC-0449, an anti-Hh agent inhibited tumor growth in tamoxifen-resistant xenograft mice, suggesting that targeting the Hh pathway alone or in combination with the PI3K pathway could potentially overcome resistance to endocrine therapy.

■ Role of receptor tyrosine kinases

Increased expression of GFR such as EGFR, HER2 or increased activation of downstream signaling molecules such as MAPK and PI3K can mediate resistance to endocrine therapy in breast cancers. Deregulation of these pathways can be due to genetic or other modifications such as overexpression of HER2, PI3KCA mutation or PTEN methylation, or alterations in upstream regulators such as AKT [26].

■ Role of HER2

Overexpression of ErbB2 is one of the best-characterized mechanisms of endocrine resistance. *HER2* amplification has been shown to confer intrinsic resistance to endocrine therapy, signifying the interaction between hormone receptors and the EGFR family [43]. Plasma membrane-associated ERs can activate HER2 through increases in second messengers such as cyclic AMP. Conversely, members of the MAPK and AKT pathway, downstream targets of HER2, can phosphorylate ER leading to ligand-independent activation [44]. Recently, the estrogen receptor coactivator MED1, has been identified as a novel interface between the HER2 and ER-α pathways, and mediates resistance to tamoxifen [45]. Increased expression of ErbB family members have been shown to be predictive for early relapse with tamoxifen in ER-positive breast cancers [46]. Therefore, combined targeting of ER and HER2 signaling has the potential to ameliorate resistance to endocrine therapy.

■ Role of FGF receptors

FGFs and their receptors (FGFRs) play essential roles in mediating cell proliferation, migration and survival [47]. Genome-wide analyses have identified multiple forms of FGFR aberrations in breast cancers.

Approximately 10% of patients with breast cancer have been shown to harbor the 8p11–12 amplicon, leading to overexpression of FGFR1 [48]. In particular, amplification of FGFR1 has been observed in 16–27% of the highly proliferative luminal B, endocrine-positive breast cancers, and has been shown to correlate with resistance to hormonal therapy leading to worse outcomes [49,50]. Interestingly, tumors with increased FGFR1 expression and ER positivity were noted to be PR negative as FGFR1 is thought

to suppress PR expression. Breast cancer cell lines with amplification of FGFR1 showed resistance to tamoxifen and this was reversed by siRNA silencing of FGFR1, suggesting that FGFR1 overexpression promotes endocrine resistance [49]. Moreover, increased expression of FGFR4 has been associated with resistance to chemotherapy, as well as endocrine therapy. Amplification of FGFR2 has also been implicated in some cases of triple-negative breast cancers [51].

Recent studies in cancer stem cells (CSCs) have shown that estrogen regulates breast CSCs through the FGF/TbX3 signaling pathway [52]. Estrogen stimulation increased the secretion of FGF family ligands and appeared to have a synergistic effect with FGF9 on increasing CSCs in MCF7 cells. FGF signaling was mediated through TbX3 expression and this effect was eliminated with the use of PD173074, a FGFR inhibitor. Interestingly, high TbX3 expression was noted in ER-positive tumors and correlated with recurrence. High TbX3 expression also correlated with poor response to chemotherapy [52]. Therefore, acquired resistance to hormonal therapy could be due to an increase in FGF/FGFR/TBX3 signaling and use of FGFR inhibitors could ameliorate this resistance.

14–3–3ζ/YWHAZ, a member of the 14–3–3 family of conserved proteins, overexpressed in ER-positive breast cancers, is associated with endocrine resistance and poor outcomes for women on tamoxifen. It plays a critical role in the regulation of FOXM1, and reduction of 14–3–3ζ levels reduces proliferation, decreases HER2 signaling and promotes apoptosis. Given the role of 14–3–3ζ in regulating growth factor signaling and mediating endocrine resistance, targeting 14–3–3ζ and FOXM1 should help overcome resistance to endocrine therapy in breast cancers [53].

■ Role of PDGF receptors

Upregulation of the PDGF receptor (PDGFR)/Abl signaling pathway has been identified in acquired resistance to estrogen deprivation with AIs. Expression of PDGFR-α and -β (tumor and stromal, respectively) was significantly correlated in pretreatment and relapse samples. High post-treatment levels of PDGFR-β were associated with a short time to relapse [54].

■ Role of cell-cycle & -survival regulators

Overexpression of MYC, cyclin D1 and E1 or decreased expression of CDK inhibitors p21 or p27 have been shown to reduce responsiveness to endocrine therapy [26]. In addition to regulating cell cycle, cyclin D1 interacts with several transcription factors, including ER-α and STAT3 [55]. Tamoxifen induces cyclin D1 binding to ER-α, which activates both STAT3 and ER-α, which means that cyclin D1 overexpression can

affect response to tamoxifen [56]. Members of the Src family of tyrosine kinases have also been implicated in endocrine resistance [57]. Resistance to tamoxifen in breast cancer cells is seen with an increase in Src kinase activity and is associated with a more aggressive phenotype [58]. In addition, estrogen and Src inhibit the activity of p27, a cell-cycle inhibitor, thus preventing cell-cycle arrest [59]. Increased expression of microRNAs miR-221 and 222 has been shown to decrease the expression of p27Kip1 leading to tamoxifen resistance [60]. Interestingly, increased expression of miR-221/miR-222 was observed in HER2-positive breast cancers resistant to endocrine therapy compared with HER2-negative tumors [61].

Autophagy is a lysosomal self-digestion pathway, where subcellular components are fused with lysosomes and then digested. Autophagy can be pro-survival or prodeath depending on the cues. A recent study using microarrays had identified heat shock 22 kDa protein (HSBP8) to block tamoxifen induced autophagy, thus leading to endocrine resistance in breast cancer [62]. dEF1, a member of the zinc finger homeodomain transcription factor family, has been shown to down regulate ER-α expression and reduce responsiveness to tamoxifen. Therefore, dEF1 is a potential therapeutic target for overcoming endocrine resistance [63]. Thus, there are various pathways for endocrine resistance that present multiple therapeutic options for amelioration.

Therapeutic approaches to overcome endocrine resistance

Despite being the mainstay of therapy in early and advanced ER-positive breast cancers, endocrine therapy eventually fails in many patients due to development of resistance. Targeting a single pathway is likely to eventually lead to resistance due to extensive crosstalk across the different pathways. Some of the drugable pathways to overcome endocrine resistance are shown in **Figure 1**. However, targeting endocrine resistance is challenging as some of the genes conferring resistance can also affect response to other agents. For instance, cyclin D1 overexpression causes resistance to gefitinib [64] and BCAR1 expression leads to adriamycin resistance [65]. BCAR1 is a Src substrate that activates proliferative and survival pathways and overexpression can reduce responses to tamoxifen [26].

While multigene panel testing such as Oncotype DX [66] or the Mammaprint [67], can classify ER-positive tumors according to the risk of recurrence and identify patients who might benefit from a combination of endocrine therapy plus chemotherapy, they do not necessarily help to identify resistance pathways and alternate treatment approaches. Therefore, therapies
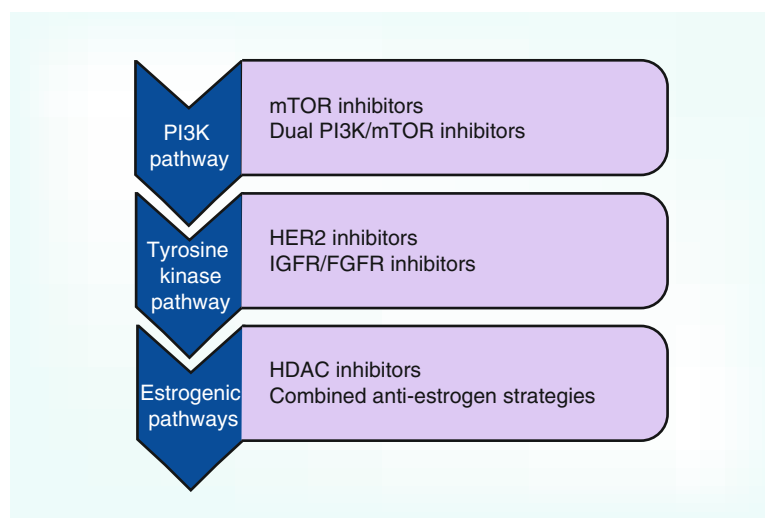
Figure 1: Pathways with drugable targets to overcome endocrine resistance.
EGFR: EGF receptor; FGFR: FGF receptor; IGFR: IGF receptor.

to overcome resistance have been the subject of intense research.

Importantly, patients who become resistant to one form of endocrine therapy often retain responsiveness to an alternate agent or combinations of anti-endocrine therapy. For instance, patients who developed resistance to nonsteroidal AIs (letrozole and anastrazole) often respond to a steroidal AI (exemestane) or fulvestrant, a SERD. This was demonstrated in a Phase III trial comparison of fulvestrant with exemestane in postmenopausal women who had disease progression after prior nonsteroidal AI therapy [68]. Fulvestrant was also shown to have significant clinical benefit after resistance to prior AI therapy [69]. Intriguingly, low-dose estradiol was shown to have clinical benefit in ER-positive, advanced breast cancers resistant to AI therapy [70]. This lack of crossresistance facilitates sequential administration of multiple hormonal agents.

A number of clinical trials have been conducted and are ongoing that target potential pathways to overcome endocrine resistance (Table 2). Activation of ErbB signaling is one of the most recognized pathways for endocrine resistance. This understanding of HER2–ER cross talk has led to the combined use of targeted agents [71]. Combination of trastuzumab and anastrazole was evaluated in the treatment of HER2- and ER-positive metastatic breast cancers in the TAnDEM trial. Combined therapy improved progression-free survival (PFS) albeit with an increase in adverse effects [72]. However, the response achieved was inferior to what would be expected with trastuzumab and chemotherapy [44]. Furthermore, combination of lapatinib and letrozole significantly improved PFS in ER-/HER2-positive breast cancers [73].

Table 2. Completed or ongoing Phase II or III clinical trials for overcoming endocrine resistance.					
Study	Phase (study name)	Regimen	Population	Outcome	Ref.
Kaufman et al.	II (TANDEM)	Anastrazole plus trastuzumab	ER/HER2 positive	Improved PFS 4.8 vs 2.4 months, albeit with increased AE with combination	[72]
Orlando et al.	II	Tamoxifen plus gefitinib	ER positive	Improved PFS 10.9 vs 8.8	[2]
Schwartzberg et al.	II	Letrozole plus lapatinib	ER/HER2 positive	Improved PFS 8 vs 3 months	[73]
Cristofanilli et al.	II	Anastrazole plus gefitinib	ER positive	Improved PFS 14.7 vs 8.4 months	[82]
Baselga et al.	III (BOLERO2)	Exemestane plus everolimus	ER positive	Improved PFS 10.6 vs 4.1 months	[75]
Bachelot et al.	II (TAMRAD)	Tamoxifen plus everolimus	ER positive	Improved CBR 61 vs 42%, improved TTP	[83]
NCT00626106	II	AMG 479 (IGF-1R mcA) plus exemestane or fulvestrant	ER positive	Results awaited	[102]
NCT01709370	II	PD0332991 (CDK 4/6 inhibitor) plus letrozole	ER positive	Ongoing	[103]
NCT01560416	II	Ganetespib (HSP90 inhibitor) plus fulvestrant	ER positive	Ongoing	[104]
NCT01594216	II	Ruxolitinib plus exemestane	ER positive	Ongoing	[105]
NCT01202591	II (GLOW)	AZD4547 (FGFR inhibitor) plus exemestane	ER positive	Ongoing	[106]
AE: Adverse event; CBR: Clinical benefit rate; ER: Estrogen receptor; FGFR: FGF receptor; PFS: Progression-free survival; TTP: Time-to-progression.					

Another important pathway involved in mediating endocrine resistance in breast cancers is the PI3K/AKT pathway. Miller *et al.* demonstrated that the development of endocrine resistance is accompanied by increased PI3K signaling and inhibition of PI3K pathway activity by the IGF-1 receptor inhibitor AEW541 or the mTOR inhibitor RAD001, or the dual PI3K/mTOR inhibitor BEZ235 suppressed tumor growth in resistant ER-positive breast cancers [74]. Treatment with temsirolimus, an mTOR inhibitor, also restored sensitivity to tamoxifen [41]. An indirect approach, using a downstream target of HER2, mTOR, was validated by the recently published BOLERO-2 trial [75]. This multicenter Phase III trial evaluated the mTOR inhibitor everolimus, in combination with exemestane, and showed an improvement in PFS in patients with ER-positive metastatic breast cancers, previously treated with a nonsteroidal AI. Everolimus was able to restore sensitivity to endocrine therapy and was generally well tolerated except for some stomatitis, fatigue and hematological side effects [75]. However, the use of letrozole with temsirolimus as first-line therapy in locally advanced or metastatic breast cancer did not show any clinical benefit [76].

In view of the importance of Src in mediating endocrine resistance, a recent Phase I study has evaluated use of dasatinib, a Src kinase inhibitor in combination with capecitabine. While the primary end point was safety, *post hoc* analyses had suggested an improvement in disease control rate and PFS in endocrine-positive patients. Further randomized trials are warranted to determine the role of Src inhibition in endocrine-refractory breast cancers [77].

In a recently reported Phase II trial, ganitumab, a monoclonal antibody against IGF-1R was evaluated in combination with exemestane or fulvestrant in previously treated hormone receptor-positive locally advanced or metastatic breast cancer patients. Median PFS did not differ between the control and experimental arms, incidentally, overall survival was worse in the ganitumab group than in the control group (hazard ratio: 1.78; p = 0.025)[78]. This calls into question the use of anti-IGFR therapy in addressing endocrine resistance. However, the heterogeneity of the patients' population and lack of molecular selection makes it difficult to draw definitive conclusions.

Considering the importance of HDAC in regulating ER expression, approaches that combine the use of HDAC inhibitors with antihormonal therapy have been evaluated. A Phase II trial of vorinostat in combination with tamoxifen in hormone-refractory breast cancers showed promising activity in reversing endocrine resistance [79]. Similarly, entinostat, another HDAC inhibitor added to exemestane improved PFS in patients with ER-positive breast cancers who progressed on a prior nonsteroidal AI [80].

FGFR1 is a potential therapeutic target as its overexpression mediates resistance to endocrine therapy, leading to early relapse and poor prognosis in luminal B-type ER-positive breast cancers [49]. Currently, FGFR inhibitors, both selective and nonselective are in clinical trials. Dovitinib (TKI258), a combined FGFR and VEGFR inhibitor, has been demonstrated to be effective in patients with FGFR1-amplified advanced breast cancers [81]. A randomized Phase II study to assess the efficacy of AZD4547, an oral FGFR inhibitor, in combination with exemestane in patients with progressive ER-positive breast cancers with FGFR1 amplification is also being carried out [101].

Future perspective

While the introduction of hormonal therapy has markedly improved survival and quality of life in patients with ER-positive breast cancers, emergence of resistance has limited improving overall clinical outcomes. The ER-signaling network is very complex with extensive crosstalk with growth factor signaling pathways, thus leading to multiple avenues of resistance. Therefore, blockade of ER-mediated signaling alone is eventually likely to lead to therapy failure. A promising strategy for the future is using rational combination therapies targeting receptor tyrosine kinases such as ErbB, FGFR and PI3K along with continued inhibition of ER. Currently, these agents have been demonstrated to have benefit in the relapsed/refractory metastatic disease when used along with endocrine therapy. However, it is uncertain as to the precise timing of the use of agents that block these alternative pathways. Studies are being carried out to see if it would be beneficial to use these agents in the adjuvant setting. Recent advances in high-throughput next-generation sequencing provide an unbiased approach to characterize putative predictive markers of resistance to endocrine therapy. Future clinical trials are likely to incorporate next-generation sequencing approaches to enroll patients based on molecular eligibility and thus assist in the design of rational combination or sequential therapies.

Executive summary

- Hormone agents (e.g., tamoxifen and letrozole) are the foundation for the treatment of estrogen receptor-positive breast cancers in the adjuvant, as well as the metastatic setting.
- The estrogen receptor-signaling network is very complex with extensive crosstalk with other signaling pathways, thus leading to multiple avenues of resistance.
- Understanding the molecular mechanisms of endocrine resistance can lead to discovery of rational combinatorial therapies.
- Inhibitors of EGF and FGF receptors, HDAC or PI3K pathways can potentially overcome resistance to endocrine therapy.
- Combination of antihormonal therapy with mTOR inhibitors has been demonstrated to improve clinical outcomes.

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■ **Websites**

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