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Academia-Pharma Intersect: Breast Cancer

Estrogen Receptor-Positive Breast Cancer: Exploiting Signaling Pathways Implicated in Endocrine Resistance

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Endocrine therapy • Estrogen receptor degrader • CDK4/6 inhibitor • Aromatase inhibitor • mTOR inhibitor

Abstract

Advancements in molecular profiling and endocrine therapy (ET) have led to more focused clinical attention on precision medicine. These advances have expanded our understanding of breast cancer (BC) pathogenesis and hold promising implications for the future of therapy. The estrogen receptor-α is a predominant endocrine regulatory protein in the breast and in estrogen-induced BC. Successful targeting of proteins and genes within estrogen receptor (ER) nuclear and nonnuclear pathways remains a clinical goal. Several classes of antiestrogenic agents are available for patients with early, advanced, or metastatic BC, including selective ER modulators, aromatase inhibitors, and a selective ER degrader. Clinical development is focused upon characterizing the efficacy and tolerability of inhibitors that target the phosphatidylinositol 3 kinase (PI3K)/akt murine thymoma viral oncogene (AKT)/mammalian target of rapamycin inhibitor (mTOR) signaling pathway or the cyclin-dependent kinase 4/6 (CDK4/6) cell cycle pathway in women with hormone receptor-positive, human epidermal growth receptor 2-negative BC who have demonstrated disease recurrence or progression. De novo and acquired resistance remain a major challenge for women with BC receiving antiestrogenic therapy. Therefore, sequential combination of targeted ET is preferred in these patients, and the ever-increasing understanding of resistance mechanisms may better inform the selection of future therapy. This review describes the intricate roles of the PI3K/AKT/mTOR and CDK4/6 pathways in intracellular signaling and the use of endocrine and endocrine-based combination therapy in BC. ***The Oncologist*** 2018;23:528–539

Implications for Practice: The foundational strategy for treating hormone receptor-positive, human epidermal growth receptor 2-negative, advanced breast cancer includes the use of endocrine therapy either alone or in combination with targeted agents. The use of combination therapy aims to downregulate cell-signaling pathways with the intent of minimizing cellular "crosstalk," which can otherwise result in continued tumorigenesis or progression through redundant pathways. This review provides the clinician with the molecular rationale and clinical evidence for these treatments and refers to evidence-based guidelines to inform the decision-making process.

Introduction

Approximately 70% of all breast cancers (BC) express the estrogen receptor (ER), progesterone receptor (PgR), or both, and such tumors are considered hormone receptor-positive (HR+) [1]. In addition to testing for the presence of ER and PgR, testing for human epidermal growth receptor 2 (HER2) protein overexpression and/or HER2 gene amplification is also performed at the time of diagnosis, and these test results aid in informing treatment decisions [2]. Molecular profiling has uncovered intrinsic subtypes in BC, including luminal A, luminal B, HER2-enriched, basal-like, and normal-like, which are associated with specific morphological and molecular features of BC [3]. Over the last decade we have also improved our understanding of intracellular signaling pathways and the cancer cell cycle, and these advances have identified promising targets for cancer therapy.

A number of classes of antiestrogenic agents are approved for patients with early, advanced, or metastatic BC, which include selective estrogen receptor modulators (SERMs), aromatase inhibitors (AIs), and a selective estrogen receptor degrader (SERD; Fig. 1) [4, 5]. However, the clinical development of combinations of antiestrogenic therapy with targeted agents that inhibit the phosphatidylinositol 3 kinase (PI3K)/akt murine thymoma viral oncogene (AKT)/mammalian target of rapamycin inhibitor (mTOR) signaling pathway or the cyclin-dependent kinase 4/6 (CDK4/6) pathway at the G_1/S checkpoint of the cell cycle is currently a key focus of clinical research in patients with

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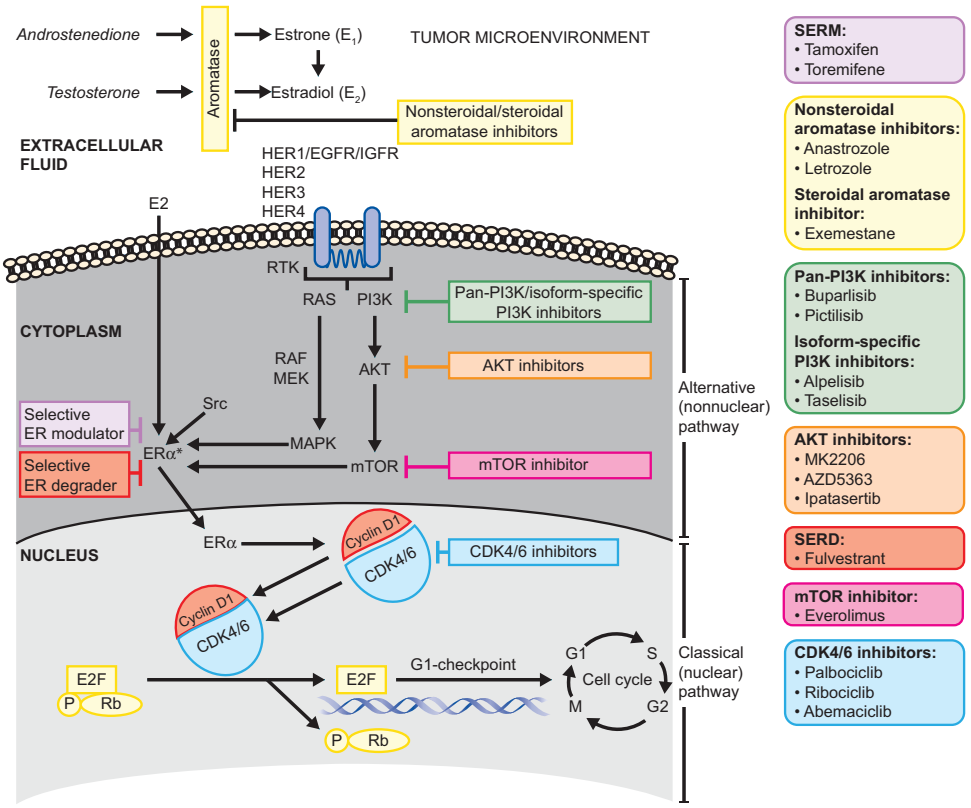

Figure 1. Critical nuclear (classical) and nonnuclear (alternative) signaling pathways implicated in endocrine resistance and targets for drugs in development. Note: Upon ligand binding, the estrogen-ER complex dimerizes and interacts with coregulator proteins and specific sequences of DNA and the estrogen response elements to promote the transcription of a wide range of genes that participate in the regulation of the cell cycle, DNA replication, cellular differentiation, apoptosis, and angiogenesis.

Abbreviations: AKT, akt murine thymoma viral oncogene; CDK4/6, cyclin-dependent kinase 4/6; E2, estradiol; E2F, *E2F* transcription factors; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERα, estrogen receptor-α; HER1, human epidermal growth receptor 1; HER2, human epidermal growth receptor 2; HER3, human epidermal growth receptor 3; HER4, human epidermal growth receptor 4; IGFR, insulin-like growth factor receptor; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated ERK-activating kinase; mTOR, mammalian target of rapamycin; P, phosphate; PI3K, phosphoinositide-3-kinase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; Rb, retinoblastoma protein; RTK, receptor tyrosine kinase; SERD, selective estrogen receptor downregulator; SERM, selective estrogen receptor modulator.

*Upon ligand binding, the estrogen-ER complex dimerizes and interacts with coregulator proteins and specific sequences of DNA and the estrogen response elements (EREs) to promote the transcription of a wide range of genes that participate in the regulation of the cell cycle, DNA replication, cellular differentiation, apoptosis, and angiogenesis.

HR+ BC who have demonstrated disease recurrence or progression [6–8]. This review describes the role of these signaling pathways and the ER in BC, the role of antiestrogens in the treatment of HR+ advanced BC, the development of resistance to antiestrogen therapy, and the use of endocrine and endocrine-based combination therapy in BC. Supporting evidence for their benefits is provided by completed phase III studies (Table 1) [9–17] and ongoing phase III studies (Table 2) [18, 19].

The Estrogen Receptor and Crosstalk

There are two functionally distinct ERs, ER-alpha (ERα, *ESR1* gene) and ER-beta (*ESR2* gene). ERα is a predominant endocrine regulatory protein in the breast and in estrogen-induced BC [20]. In this review, references to the ER pertain to ERα/*ESR1*. Estrogen binds to the ER with high affinity and specificity and functions through two main types of pathways, the classical (or nuclear) pathway and the alternative (nonnuclear) pathway (Fig. 1) [21]. Successful targeting of genes within these nuclear and nonnuclear pathways remains an important clinical goal. Along the classical pathway, the estrogen-ER complex dimerizes upon ligand binding and interacts with coregulator proteins and specific sequences of DNA called estrogen responsive elements. These interactions promote the transcription of a wide range of genes that participate in the regulation of the cell cycle, DNA replication, cellular differentiation, apoptosis, and angiogenesis [7, 21].

The engagement of the ER with estrogen through nonnuclear pathways originates in the cytoplasm to trigger coregulator growth factor and G-protein coupled signaling (Fig. 1). Coregulators in the nonnuclear pathways include receptors (e.g., insulin-like growth factor-1 receptor, fibroblast growth factor receptor [FGFR], HER2), and kinases (e.g., mitogen-activated protein kinases, receptor tyrosine kinase, PI3K, AKT, mTOR, Src, and CDK). Because the ER can also be activated through ligand-independent mechanisms, multiple opportunities exist for crosstalk between the ER, growth factors, and protein kinases, which can activate or modulate ER activity [7, 21].

Estrogen-Mediated Effects on the Cell Cycle

Progression through the cell cycle is controlled by the binding of cyclins and the ensuing dimerization of CDKs, which are synthesized and degraded at specific points throughout the cell cycle (Fig. 1). Estrogen is instrumental to this process by

Table 1. Completed phase III studies of endocrine-based combination therapies for advanced or metastatic breast cancer

Study acronym Clinicaltrials.gov identifier	Patients, *n*	Patient criteria	Treatment arms	Median PFS/TTP (95% CI), months Hazard ratio	Median OS (95% CI), months Hazard ratio	ORR, % (95% CI)
CDK4/6 inhibitor combinations						
First-line therapy						
PALOMA-2 NCT01740427 [9]	666	Postmenopausal ER+, HER2− Advanced disease No prior treatment for advanced disease	Palbociclib + letrozole	24.8 (22.1–NR) 0.58 (0.46–0.72); $p < .001$	NR	42.1 (37.5–46.9)
			Letrozole + PBO	14.5 (12.9–17.1)	NR	34.7 (28.4–41.3)
MONALEESA-2 NCT01958021 [10, 11]	668	Postmenopausal ER+, HER2− Advanced/metastatic disease No prior systematic therapy	Ribociclib + letrozole	NR (19.3–NR)[a] 0.56 (0.43–0.72); $p < .001$	NR[b]	40.7 (35.4–46.0)[a]
			Letrozole + PBO	14.7 (13.0–16.5)[a]	NR[b]	27.5 (22.8–32.3)[a]
MONARCH-3 NCT02246621 [74]	493	Postmenopausal HR+, HER2− Locoregional recurrent or metastatic disease No prior systematic therapy	Abemaciclib + anastrozole or letrozole	NR 0.54 (0.41–0.72); $p < .001$	NR[c]	48.2 (42.8–53.6)
			PBO + anastrozole or letrozole	14.7	NR[c]	34.5 (27.3–41.8)
Second-line therapy						
MONARCH-2 NCT02107703 [12]	669	Pre- or postmenopausal ER+, HER2− Advanced disease Progressed during neoadjuvant or adjuvant endocrine ET, ≤12 months from the end of adjuvant ET, or while receiving first-line ET for metastatic disease	Abemaciclib + fulvestrant	16.4 0.55 (0.449–0.681); $p < .001$	NR	48.1
			Fulvestrant + PBO	9.3	NR	21.3
PALOMA-3 NCT01942135 [13, 14]	521	Pre- or postmenopausal HR+, HER2− Relapsed or progressed during prior ET	Palbociclib + fulvestrant	11.2 0.50 (0.40–0.62); $p < .0001$	NR	19 (15.0–23.6)
			Fulvestrant + PBO	4.6 (3.5–5.6)	NR	9 (4.9–13.8)
mTOR inhibitor combinations						
BOLERO-2 NCT00863655 [15]	724	Postmenopausal HR+ Locally advanced/ metastatic disease Progression after anastrozole or letrozole	Everolimus + exemestane	7.8	NR	12.6 (9.8–15.9) $p < .001$
			Exemestane + PBO	3.2 0.45 (0.38–0.54); $p < .001$		1.7 (0.5–4.2)

(continued)

Table 1. (continued)

Study acronym ClinicalTrials.gov identifier	Patients, *n*	Patient criteria	Treatment arms	Median PFS/TTP (95% CI), months Hazard ratio	Median OS (95% CI), months Hazard ratio	ORR, % (95% CI)
PI3K inhibitor combinations						
BELLE-2 NCT01610284 [16]	1,147	Postmenopausal HR+, HER− Locally advanced or MBC Progression on or after AI therapy	Buparlisib + fulvestrant	6.9 (6.8–7.8)	NR	11.8
			Fulvestrant + PBO	5.0 (4.0–5.2) 0.78 (0.67–0.89); $p < .001$	NR	7.7
BELLE-3 NCT01633060 [17]	432	Postmenopausal HR+, HER2− Metastatic disease Prior AI therapies Progression on combination of mTOR inhibitor and ET	Buparlisib + fulvestrant	3.9 (2.8–4.2)	NR	7.6 (4.8–11.3)
			Fulvestrant + PBO	1.8 (1.5–2.8) 0.67 (0.53–0.84); $p < .001$		2.1 (0.4–6.0)

[a]The PFS and ORR data appear in the Hortobagyi 2016 article [10]; however, the more recent Hortobagyi 2017 ASCO abstract [11] reports 24-month PFS rates as 54.7% vs. 35.9% (without citing 95% CI or *p* value).
[b]The OS data remain immature, but the 2017 abstract [11] mentions 15.0% vs. 19.8% of patient deaths (hazard ratio 0.746; 95% CI, 0.517–1.078; $p = .059$).
[c]The OS data remain immature, but the 2017 publication [74] mentions 9.8% vs. 10.3% of patient deaths (hazard ratio 0.97).
Abbreviations: AI, aromatase inhibitor; CDK4/6, cyclin-dependent kinase 4/6; CI, confidence interval; ER+, estrogen receptor-positive; ET, endocrine therapy; HER2−, human epidermal growth receptor 2-negative; HR+, hormone receptor-positive; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; NR, not reported/reached; ORR, overall response rate; OS, overall survival; PBO, placebo; PFS, progression-free survival; PI3K, phosphoinositide-3-kinase; TTP, time to progression.

facilitating the G_1 to S phase transition by means of the activation and binding of cyclin D1 to dimerized CDK4/6 [6, 22, 23]. Dysregulation of the cyclin D1/CDK4/6 pathway is reported to be an early and essential gateway for breast tumorigenesis because the overexpression of cyclin D1 has been implicated in the development of BC [24–26], several other solid tumors, and hematologic malignancies [26, 27]. Amplification of the gene for cyclin D1, *CCND1,* occurs in many human BC tumors, including 29% of luminal A cancers, 58% of luminal B cancers, and 38% of the HER2-expressing molecular subtypes; overexpression of CDK4 occurs in 14%, 25%, and 24% of these subtypes, respectively [28].

Endocrine Therapy Action on Estrogen Receptor and Tumor Microenvironment

Antiestrogenic therapies used to treat HR+ BC may target either estrogen production or the estrogen receptor (Fig. 1) [20]. In existing treatment guidelines, the use of monotherapy or combination approaches may vary based on prior adjuvant endocrine therapy (ET) exposure status and whether relapse or recurrence occurred before or after 12 months since adjuvant treatment. Endocrine therapies currently utilized in the first- or second-line for estrogen-positive BC include the AIs, anastrozole, letrozole, and exemestane; the SERD, fulvestrant [2, 4, 5]; and the SERMs, tamoxifen and the chlorinated derivative toremifene [29]. High-dose estrogen (ethinyl estradiol), progestin (megestrol acetate), and the androgen, fluoxymesterone, are recommended as third- and later-line therapy [5]. In addition, several targeted therapies have become available for use either as monotherapy or in combination with ET, including other CDK4/6 inhibitors (e.g., abemaciclib, palbociclib, and ribociclib) and the mTOR inhibitor, everolimus.

The downstream effects of SERMs binding the ER are tissue-specific and may differ among the various agents, for example, agents that act as antagonists in BC tissue and, alternatively, as partial agonists in other tissues [30]. These disparate properties are also demonstrated by other SERMs, such as raloxifene, which is used to reduce the risk of BC in postmenopausal patients with osteoporosis or who are otherwise at risk of invasive breast cancer and also as a treatment for osteoporosis [31].

Aromatase inhibitors reduce the production of estrogen by inhibiting the aromatase enzyme activity in peripheral tissues and within the tumor microenvironment (Fig. 1). Exemestane is a steroidal molecule that irreversibly and covalently binds to aromatase [32]. The nonsteroidal agents, anastrozole and letrozole, reversibly bind aromatase [33]. Preclinical studies suggested that letrozole alone was superior to tamoxifen, and no additional benefits were evident for the combination treatment; studies with anastrozole in combination with tamoxifen reported findings similar to those with letrozole [34, 35]. Lastly, no advantage of the atamestane (a steroidal AI) plus toremifene combination over letrozole monotherapy was observed in a phase III study of postmenopausal women with advanced receptor-positive BC [36]. Consequently, there has not been a strong rationale to further explore tamoxifen or toremifene in combination with AIs as a first-line doublet.

The SERD fulvestrant is a 7α-alkylsulphinyl analogue of 17β-estradiol [37] that competitively inhibits the binding of estradiol to the ER and binds with similar affinity as estradiol (50% inhibitory concentration [IC_{50}], 0.89), and a much higher

Table 2. In-progress phase III studies of endocrine-based combination therapies for advanced/metastatic breast cancer

Clinicaltrials.gov identifier (acronym)	Comparators	Patient and enrollment criteria	Primary endpoint Secondary efficacy and QoL endpoints	Start date Est. study completion Est. primary completion[a]
NCT02763566 (MONARCH plus)	Abemaciclib + anastrozole or abemaciclib + letrozole vs. Abemaciclib + fulvestrant or abemaciclib + PBO	Postmenopausal HR+, HER2− Locoregional recurrent disease not amenable to resection or radiation therapy or measurable disease	PFS OS, CR, PR, ORR, DoR, DCR, CBR, EORTC QLQ-C30	December 2016 January 2020
NCT02422615 (MONALEESA-3)	Ribociclib + fulvestrant Fulvestrant + PBO	Men and postmenopausal women ER+ and/or PgR+, HER2− Advanced/metastatic disease either newly diagnosed, relapsed >12 months of completing neoadjuvant/adjuvant ET for early disease, relapsed on/within 12 months of neoadjuvant/adjuvant treatment of early disease, relapsed >12 months after completing adjuvant ET and then progression after one line of ET for advanced/metastatic disease, or newly diagnosed advanced/metastatic with progression after one line of ET	PFS OS, ORR, time to deterioration of ECOG performance status in one category of the score, time to 10% deterioration in the global health status/QoL scale score of the EORTC QLQ-C30, CBR, TTR, DoR	July 2015 February 2020
NCT02340221 (SANDPIPER) [18]	Taselisib + fulvestrant Fulvestrant + PBO	Postmenopausal ER+ Locally advanced/metastatic disease Recurrence/progression during/after AI therapy Enriched for *PIK3CA*-mutant tumors	PFS OS, ORR, CBR, DoR	April 2015 June 2018
NCT02437318 (SOLAR-1) [19]	Fulvestrant + alpelisib Fulvestrant + PBO	Men and postmenopausal women HR+, HER2− Advanced breast cancer Identified *PIK3CA* status Relapsed during or within 12 months of neoadjuvant or adjuvant ET with no or one line of ET for metastatic disease or newly diagnosed with progression during or after one line of ET, recurrence/progression during or after AI therapy	PFS OS, ORR, CBR, time to deterioration in ECOG status, time to 10% deterioration in the global health status/QoL scale score of the EORTC QLC-C30	July 2015 February 2020 January 2018

[a]If different from estimated study completion.

Abbreviations: AI, aromatase inhibitor; CBR, clinical benefit rate; CR, complete response; DCR, disease control rate; DoR, duration of response; ECOG, Eastern Cooperative Oncology Group; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30; ER+, estrogen receptor-positive; est., estimated; ET, endocrine therapy; HER2−, human epidermal growth receptor 2-negative; HR+, hormone receptor-positive; ORR, overall response rate; OS, overall survival; PBO, placebo; PFS, progression-free survival; PgR+, progesterone receptor-positive; PR, partial response; QoL, quality of life; TTR, time to response.

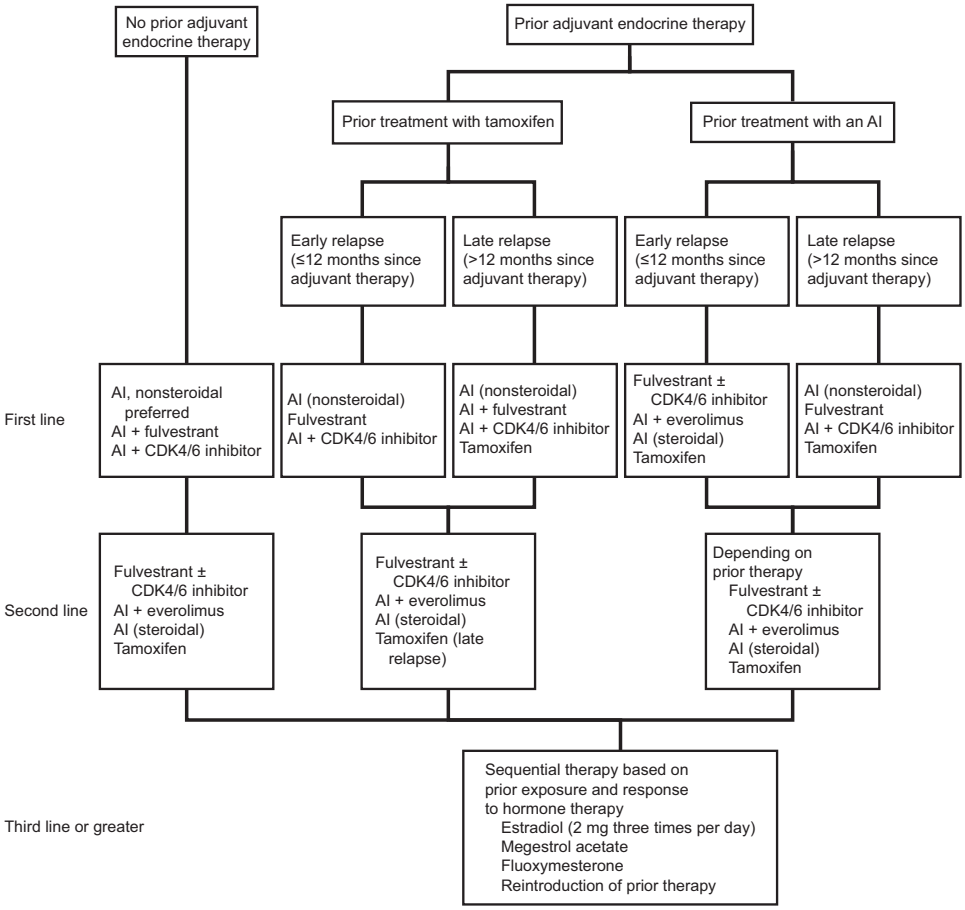

Figure 2. Treatment algorithm for endocrine therapy for hormone receptor-positive metastatic breast cancer. Treatment recommendations for premenopausal patients include ovarian suppression. In the setting of patients with no prior adjuvant endocrine therapy, tamoxifen may be considered for premenopausal women. (Adapted from Rugo et al. [5] with permission from the American Society of Clinical Oncology.) Note: Treatment alternatives include an AI with or without a CDK4/6 inhibitor or fulvestrant with or without a CKD4/6 inhibitor.

Abbreviations: AI, aromatase inhibitor; CDK4/6, cyclin-dependent kinase 4/6.

affinity than does tamoxifen (IC_{50}, 0.19–0.25) [38, 39]. The binding of fulvestrant to ER monomers exerts several effects: inhibition of ER dimerization, inactivation of subunit transcription activating factor 1 and activating factor 2, attenuation of the translocation of ER to the nucleus, accelerated ER degradation, and ER downregulation (Fig. 1) [40]. The activity of fulvestrant is characterized by pure ER antagonism with exclusively antiestrogenic effects on breast tissue, resulting in the inhibition of estrogen-dependent breast tumor cell proliferation [38, 40, 41]. Fulvestrant is known for its effects on the cell during the G_1/S phase transition, by increasing the proportion of cells in G_0/G_1 and decreasing the proportion of cells undergoing continued DNA synthesis [38].

The activity of fulvestrant is characterized by pure ER antagonism with exclusively antiestrogenic effects on breast tissue, resulting in the inhibition of estrogen-dependent breast tumor cell proliferation

The initial U.S. Food and Drug Administration (FDA) approval of fulvestrant was for a monthly 250-mg dose administered by intramuscular injection [42, 43]. Some of the early fulvestrant studies also used a 500-mg loading dose. Subsequently, however, the CONFIRM study provided the basis for approval of the 500-mg dose by comparing fulvestrant 250 mg with 500 mg and demonstrating improved efficacy and a similar safety profile for the higher dose [44, 45]. The FALCON study conducted in postmenopausal ET-naïve patients with HR+, HER2-negative (HER2−) BC found that a 500-mg fulvestrant loading schedule was associated with a significantly longer progression-free survival (PFS; 16.6 vs. 13.8 months; $p = .049$; primary endpoint) and provided significantly longer duration of response (11.4 vs. 7.5 months; $p = .037$) compared with anastrozole [4]. Updated analysis of this trial indicates that fulvestrant is also associated with maintenance of health-related quality of life in this population [46]. In August 2017, the indication of fulvestrant was expanded to include treatment of HR+, HER2−, advanced BC in postmenopausal women not previously treated with endocrine therapy [47].

Antiestrogenic Effects on Signaling Pathways and Development of Resistance to Antiestrogenic Therapy in Breast Tumor Cells

Development of endocrine resistance has been linked to overexpression and/or amplification of a number of genes in growth

factor pathways, including those mediated by HER2, human epidermal growth receptor 3, epidermal growth factor receptor, FGFR-1, and insulin-like growth factor receptor-1 (Fig. 1) [48–51]. These proteins signal along the PI3K/AKT/mTOR pathway [1]. Overexpression of HER2, which occurs in approximately 15% to 20% of all BC [52], mediates cell growth and survival through activation of its downstream mediators, PI3K/AKT/mTOR and rat sarcoma/rapidly accelerated fibrosarcoma/mitogen-activated ERK-activating kinase/mitogen-activated protein kinase. Aberrant activation of the PI3K/AKT/mTOR pathway, a key pathway axis in the signaling network, is associated with ligand-independent ER activation and subsequent activation of downstream pathways without traditional binding or regulation of estrogen [52].

The *PIK3CA* gene is mutated in as many as half of breast tumors [53–55], with mutation status being highly concordant between primary and metastatic tumors [56, 57]. Alterations in the PI3K/AKT pathway, including *AKT* mutations and loss of phosphatase and tensin homolog (*PTEN)*, occur in more than 70% of breast tumors [58]. The mTOR protein complex is a major downstream target of AKT [59].

A number of studies have implicated cyclin D1 and cyclin-dependent kinases in endocrine resistance. In vitro studies demonstrated that HR+ BC cells induced to overexpress cyclin D1 continued to grow in the presence of tamoxifen [60], and cyclin D1 has been shown to be essential for the proliferation of tamoxifen-resistant cells [61]. Breast tumor cells that develop resistance to ET maintain activation of cyclin D1 and the subsequent phosphorylation of retinoblastoma protein (Fig. 1) [62]. Higher levels of cyclin D1 mRNA in HR+ primary tumors among patients receiving tamoxifen have been associated with worse outcomes, including significantly shorter time to recurrence ($p = .025$), time to metastasis ($p = .019$), and overall survival (OS; $p = .025$) [63].

Adjuvant AI therapy appears to select for *ESR1* mutations under the stress of estrogen deprivation, in which there is genetic intratumoral heterogeneity and clonal diversity. In contrast, treatment with fulvestrant does not select for *ESR1* mutations conferring constitutive activation of ERα

Mutations in *ESR1* have also been linked to acquired endocrine resistance, with the most common *ESR1* mutations affecting one of two residues in the ER ligand-binding domain [1]. These *ESR1* mutations confer constitutive or estrogen-independent activation of the ER and resistance to AI therapy [64, 65]. *ESR1* mutations are rarely found in treatment-naïve patients and are hardly ever the cause of primary resistance [1]. Instead, *ESR1* mutations associated with endocrine resistance are found in patients with metastatic disease who have been treated with AIs [65–67]. Adjuvant AI therapy appears to select for *ESR1* mutations under the stress of estrogen deprivation, in which there is genetic intratumoral heterogeneity and clonal diversity [68, 69]. In contrast, treatment with fulvestrant does not select for *ESR1* mutations conferring constitutive activation of ERα [67]. More work is ongoing to clarify the impact of *ESR1* mutations on response to therapy, with future trials incorporating serial cell-free DNA sampling to quantify and follow *ESR1* mutational burden and correlate with response to therapy.

Endocrine Therapy Options to Manage Resistance

Although ETs are a common first-line treatment in advanced or metastatic breast cancer (MBC), resistance inevitably develops [5]. A deeper understanding of the fundamental mechanisms of ET resistance has led to the development of treatment options and strategies, as well as a greater awareness of how to better utilize existing agents.

Whereas some patients may develop resistance to ET with one agent class, a response to treatment may occur with exposure to another class. Sequential ET is preferred in postmenopausal women with HR+, HER2− MBC [2, 5]. Guidelines currently recommend AIs with the CDK4/6 inhibitors, palbociclib or ribociclib, or fulvestrant (either as monotherapy or in combination with anastrozole) as a first-line ET option. As a second-line ET option, fulvestrant in combination with palbociclib or abemaciclib is recommended for patients with prior adjuvant ET exposure or patients who received ET in the metastatic setting. Everolimus may also be administered with exemestane upon disease progression in women who are refractory to nonsteroidal AIs. The ET combination studies described below have been designed with impactful clinical outcomes in mind, which may circumvent resistance challenges (Fig. 2, Table 1).

Targeted CDK4/6 Inhibitors

First-Line Regimens

Palbociclib (PD0332991) is a highly selective and potent inhibitor of CDK4/6 (Fig. 1) [70]. This oral agent is now indicated in combination with any AI as first-line endocrine-based treatment of patients with HR+, HER2−, locally advanced or metastatic BC and in combination with fulvestrant in women with disease progression after ET, based on data indicating that it improves PFS compared with ET alone.

PALOMA-1 evaluated palbociclib plus letrozole in patients with HR+, HER2−, advanced BC. A second cohort required cancer to have cyclin D1 amplification and/or loss of p16 [71]. Treatment with palbociclib and letrozole was associated with a significantly longer PFS versus letrozole monotherapy (20.2 vs. 10.2; hazard ratio 0.49, $p < .001$) [71]; however, the difference in OS was not significant (37.5 vs. 34.5 months; hazard ratio 0.84, $p = .28$) [72]. In the confirmatory phase III PALOMA-2 study, conducted in postmenopausal patients without prior systemic therapy for advanced BC, palbociclib plus letrozole was superior to letrozole alone in terms of median PFS (24.8 vs. 14.5 months; hazard ratio 0.58, $p < .001$), objective response rate (ORR; 42.1% vs. 34.7%; $p = .06$), and confirmed ORR in patients with measurable disease (55.3% vs. 44.4%; $p = .03$; Table 1). The most common adverse events (AEs) with the combination were neutropenia, leukopenia, fatigue, nausea, arthralgia, and alopecia [9].

Ribociclib (LEE011) became the second CDK4/6 inhibitor to receive FDA approval as a first-line treatment for HR+, HER2−, advanced BC in combination with any AI in postmenopausal women [73], based on the results of the MONALEESA-2 study (Table 1) [10, 11]. After a median follow-up of 26.4 months, PFS (the primary endpoint) significantly favored the ribociclib plus letrozole group over the letrozole-only group, with 24-month

PFS rates of 54.7% and 35.9%, respectively. The OS data remain immature, with 15% of patient deaths in the combination arm versus 19.8% in the letrozole-only arm (hazard ratio 0.746; $p = .059$) [11]. The most common AEs with ribociclib were neutropenia, nausea, infections, fatigue, and diarrhea [10]. Furthermore, a preplanned interim analysis of the ongoing MONARCH-3 trial has found that abemaciclib in combination with an AI met its primary endpoint of improved PFS versus AI plus placebo (hazard ratio 0.54; 95% confidence interval [CI], 0.41–0.72; $p = .000021$) in women with HR+, HER2−, advanced BC [74].

Second-Line Regimens

Palbociclib in combination with fulvestrant in CDK4/6 inhibitor-naïve women with disease progression after ET represents a second-line option in HR+ advanced or metastatic BC [5, 75]. Studies compared the combination of palbociclib plus fulvestrant versus fulvestrant alone in patients with HR+, HER2− metastatic disease with progression after prior ET (including tamoxifen and AIs) [13]. In the PALOMA-3 study, patients receiving palbociclib plus fulvestrant demonstrated significantly longer PFS compared with fulvestrant plus placebo (11.2 vs. 4.6 months; hazard ratio 0.50, $p < .001$) [14]. All subgroup analyses of PFS favored palbociclib plus fulvestrant over fulvestrant alone. Findings from the PALOMA-3 study (Table 1) were the basis of FDA and European Union approval of the combination of palbociclib with fulvestrant as a second-line treatment option.

Studies are ongoing with the CDK4/6 inhibitor, abemaciclib (LY2835219), either alone or in combination with ET, for patients with HR+, HER2− advanced or metastatic BC who have relapsed after ET [76], including studies to treat brain metastases [12, 77, 78]. The efficacy of abemaciclib monotherapy was demonstrated in the phase II MONARCH-1 study in heavily pretreated women with HR+, HER2− metastatic disease with progression during or after ET and one or two prior chemotherapy regimens administered for advanced-stage disease [79]. At the 12-month analysis, ORR was 19.7%, the clinical benefit rate (CBR) was 42.4%, median PFS was 6 months, and OS was 17.7 months [79]. In MONARCH-2, a phase III study in patients with HR+, HER2− MBC who experienced relapse or progression after ET, the addition of abemaciclib to fulvestrant demonstrated a significant improvement in PFS (16.4 months) compared with fulvestrant alone (9.3 months). The ORR among patients treated with abemaciclib plus fulvestrant was 48.1% compared with 21.3% in the control arm (Table 1) [12]. Based on these results, abemaciclib was recently approved in combination with fulvestrant for women with HR+/HER2− advanced or metastatic BC with disease progression after ET and as monotherapy after ET and prior chemotherapy in the metastatic setting [80].

mTOR Inhibitors

Everolimus is an analog of rapamycin that inhibits the mTOR complex and leads to a variety of downstream effects, including blocking cell growth, angiogenesis, and dysregulation of cellular metabolism (Fig. 1) [81]. Everolimus is approved in the U.S. for use in combination with exemestane in postmenopausal women with HR+, HER2− advanced BC who demonstrated progression after failure of treatment with anastrozole or letrozole [82]. Approval was based on the findings of the BOLERO-2 study, during which patients received everolimus plus exemestane or exemestane plus placebo. The majority of patients (80%) had received prior therapy, including tamoxifen (48%), fulvestrant (17%), or chemotherapy (26%) [15]. In the final analysis, the PFS for everolimus plus exemestane was significantly greater than for everolimus plus placebo (7.8 months vs. 3.2 months; hazard ratio 0.45, $p < .001$; Table 1) [15]. In this study, the most common AEs in the combination arm were stomatitis, rash, fatigue, diarrhea, nausea, decreased appetite, weight loss, and cough; however, with everolimus alone, patients experienced mainly nausea and fatigue [15].

PI3K Inhibitors

The PI3K signaling pathway is an active regulator of cellular processes, including cell proliferation, growth, survival, migration, and metabolism. Hyperactivation of the PI3K/AKT pathway occurs frequently in human cancers, which makes it a therapeutic target of particular interest [58, 83, 84]. Oral PI3K inhibitors in development for advanced or metastatic BC in combination with antiestrogen therapies (Fig. 1) include the selective isoform-specific PI3Kα inhibitors taselisib (GDC-0032) [85] and alpelisib (BYL719) [86]. Two pan-PI3K inhibitors (Fig. 1), buparlisib (BKM120) [16, 87] and pictilisib (GDC-0941) [88, 89], were under investigation, but poor toxicity led to the discontinuation of further development.

AKT Inhibitors

The investigational drug MK-2206 is a potent and specific allosteric inhibitor of the AKT family in vitro and displayed no activity against more than 250 protein kinases during in vivo testing (Fig. 1) [90]. A phase I study was conducted to determine the recommended phase II treatment dose and activity of MK-2206 in combination with anastrozole and with fulvestrant in postmenopausal patients with HR+ MBC [91]. The CBR was 36.7%, the median time to progression was 5.8 months, and the ORR was 15.4%. The activity of these combinations was lower than observed with endocrine monotherapy. Possible reasons included the low dose used because of treatment-associated rash, possible mismatch between the mechanism of action of MK-2206 and tumor resistance mechanisms of the study participants because confirmation of AKT mutation was not performed, and differences in tumor cell behavior in the clinical and preclinical settings [91].

The investigational agent AZD5363 is a potent inhibitor of the AKT family that in combination with fulvestrant showed synergy in an HR+ patient-derived xenograft model and delayed tumor progression after treatment ended [92]. Preclinical studies of AZD5363 oral dosing resulted in significant inhibition of estrogen-responsive human breast xenografts [93]. The phase I study FAKTION (NCT01992952) will assess AZD5363 in combination with fulvestrant in a subgroup of *AKT1* (*E17K*) mutation-positive patients. Completed early clinical studies have reported responses in patients with and without *AKT1* (*E17K*) mutations [94, 95].

Ipatasertib is an AKT inhibitor currently in phase II studies for triple-negative breast cancer and phase III studies for prostate cancer. In the phase II LOTUS study, ipatasertib or placebo was added to paclitaxel for first-line treatment of women ($n = 124$) with locally advanced or metastatic triple-negative breast cancer [96]. After a median follow-up of 10.4 months in the ipatasertib group and 10.2 months in the placebo group, median PFS was 6.2 months with ipatasertib and 4.9 months with placebo (hazard ratio 0.60, 95% CI, 0.37–0.98; $p = .037$). In patients with PTEN-low tumors ($n = 48$), PFS was 6.2 months

with ipatasertib versus 3.7 months with placebo (hazard ratio 0.59, 95% CI, 0.26–1.32; $p = .18$) [96]. These results suggest that further development is warranted.

Future Directions in Endocrine Therapy Research

In vitro and preclinical data for various solid tumors support combining CDK4/6 inhibition with PI3K inhibition [97]. In a preclinical mouse model of PI3K inhibitor-resistant BC, ribociclib plus pictilisib or alpelisib showed synergistic activity. The most active combination to date in vivo consists of ribociclib plus buparlisib or alpelisib and letrozole or fulvestrant. Triplet combinations using CDK4/6 and PI3K inhibitors and endocrine therapies are being evaluated in a few early-stage clinical studies. These include the phase I study comparisons of fulvestrant plus ribociclib with and without buparlisib (NCT02088684), a study of letrozole plus ribociclib plus alpelisib (NCT01872260), and a study of ribociclib plus everolimus plus exemestane (NCT01857193). In other advances, researchers have exploited similarities in the ATP-binding sites of PI3K and mTOR to create dual inhibitors that target all isoforms of PI3K and also both mTOR complexes [98, 99]. It is hoped that this strategy may eliminate the potential for molecular crosstalk loops that activate AKT when mTOR is inhibited. For example, the PI3K inhibitor, gedatolisib, is currently being investigated in a phase I dose-escalation study (NCT02626507) with palbociclib and fulvestrant in the neoadjuvant setting for previously untreated patients with ER-positive, HER2− BC.

There are also new classes of agents that are being combined with ET in patients with HR+ disease. This includes FGFR inhibitors that can reverse endocrine resistance in BC cells. The FGFR inhibitor AZD4547 is currently being evaluated in combination with letrozole or anastrozole in patients with disease progression on these AIs [100]. Bromodomain and extraterminal (BET) proteins reduce ER expression and downregulate ER-dependent gene expression. The BET inhibitor GSK626762 is being evaluated in combination with fulvestrant in patients with ER-positive advanced or metastatic BC [101]. Other SERDs are also under investigation. Elacestrant (RAD1901) is a SERD that has shown antitumor activity in multiple ER-positive BC patient-derived xenograft models [102] and is under investigation both for metastatic BC and for menopausal vasomotor symptoms. GDC-0810 is being studied as monotherapy and in combination with palbociclib and/or a luteinizing hormone-releasing hormone agonist (NCT01823835), as well as in a phase II study versus fulvestrant (NCT02569801).

Several phase III studies investigating dual combinations with fulvestrant therapy are ongoing (Table 2). These studies include SANDPIPER, which is evaluating fulvestrant plus taselisib [18], and SOLAR-1, which is assessing fulvestrant plus alpelisib versus fulvestrant plus placebo [19]. MONALEESA-3 (fulvestrant plus ribociclib vs. ribociclib plus placebo) is an ongoing CDK4/6 study expected to complete in February 2020. Although all patients with MBC ultimately progress on fulvestrant, the exact mechanisms of progression are currently not well characterized. It is expected that these mechanisms will be further elucidated as our understanding of resistance grows. However, fulvestrant combinations may potentially provide a longer course to the emergence of ER resistance.

Adjuvant therapy is another area under intense investigation. There are several ongoing placebo-controlled phase III studies evaluating the efficacy and safety of adding CDK4/6 inhibitors to standard adjuvant ET. These adjuvant studies include PALLAS, which is investigating the addition of palbociclib to standard adjuvant ET for patients with HR+, HER2−, early BC (NCT02513394); monarchE, which is evaluating abemaciclib plus adjuvant ET in patients with HR+, HER2−, high-risk, node-positive, early-stage BC (NCT03155997); and earLEE-1 (NCT03078751) and earLEE-2 (NCT03081234), both of which will evaluate the efficacy and safety of ribociclib plus adjuvant ET in patients with high-risk and intermediate-risk early BC, respectively. The mTOR inhibitor everolimus is being evaluated as adjuvant therapy in a phase III study in combination with ET in patients with high-risk, HR+, HER2− BC (NCT01674140).

Conclusion

Molecular profiling of breast tumors is providing a better understanding of ET resistance, which will assist in the development of agents with new targets in order to deliver precision medicine to patients with HR+, HER2− MBC. Despite this improved insight, resistance to ET is a hallmark of relapse and progression in MBC. Some patients may retain tumor cells with functional hormone receptors, and many breast tumor cells may develop resistance to ET.

Combination therapies that efficaciously inhibit tumor growth by affecting cell cycle modulators and regulators of key signaling pathways have recently been approved, and new agents with antiestrogenic effects on the intracellular pathways are being tested. Endocrine agents also continue to be explored as monotherapy. Endocrine therapy is generally well tolerated and associated with low toxicity; however, increased risk of toxicity may be noted in patients with advanced BC when therapy is combined with certain targeted therapy. Implementation of molecular profiling, histopathology, treatment modalities, and general patient well-being in clinical practice all have a role in patient outcomes.

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For Further Reading:

Kathleen I. Pritchard, Stephen K. Chia, Christine Simmons et al. Enhancing Endocrine Therapy Combination Strategies for the Treatment of Postmenopausal HR+/HER2− Advanced Breast Cancer. ***The Oncologist*** 2017;22:12–24; first published on November 18, 2016.

Implications for Practice:

Emerging data show that new endocrine therapy (ET) combinations can improve progression–free and overall survival outcomes in patients with hormone receptor–positive, HER2–negative (HR+/HER−) advanced breast cancer. Level 1 evidence supports consideration of dual ET regimens, particularly in ET-naïve patients, or palbociclib plus letrozole as first-line therapy, as well as the addition of mTOR or CDK4/6 inhibitors to established ET in the second-line setting and in select first-line patients. Some combinations are associated with increased risk of class-specific toxicities that will require individualized risk stratification, earlier and more rigorous agent-specific monitoring, and patient education. Recent data on a noninvasive biomarker assay that predicts response to a phosphoinositide 3–kinase inhibitor demonstrates the feasibility of this minimally invasive technique as an alternative to traditional tissue analysis.