New Targets in Endocrine-Resistant Hormone Receptor–Positive Breast Cancer

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Abstract: Endocrine-based treatments are the backbone of initial therapy for advanced hormone receptor–positive breast cancers. Developing new therapeutic strategies to address resistance to endocrine therapy is an area of active research. In this review, we discuss targeted therapies that are currently the standard of care, as well as agents that are at present under investigation as potential treatments for advanced hormone receptor–positive breast cancer.

Keywords
Endocrine resistance, hormone receptor–positive breast cancer, targeted therapy

Introduction

Hormone receptor–positive (HR+) breast cancers are the most commonly identified subtype of breast cancer, accounting for about 70% of early and de novo metastatic breast cancer diagnoses.[1,2] Estrogen is the main driver of cancer cell proliferation in HR+ breast cancer. Upon binding with estrogen, the estrogen receptor (ER) acts as both a direct transcription factor and a regulator of other transcription factors to drive cell proliferation.[3] Therefore, a key component in the initial treatment of metastatic HR+ breast cancer is estrogen deprivation. In the treatment-naive state, most advanced HR+ breast cancers are sensitive to estrogen blockade with either an aromatase inhibitor (AI; eg, letrozole, anastrozole, or exemestane) for postmenopausal women or an AI plus ovarian suppression for premenopausal women.

Resistance to endocrine therapy is an inevitable development, however, and may be acquired or primary. Acquired resistance is defined as disease progression beyond 2 years of adjuvant endocrine therapy or after at least 6 months of endocrine therapy in the setting of advanced breast cancer; primary resistance is defined as disease progression within 2 years of adjuvant endocrine therapy or after less than 6 months of endocrine therapy in the advanced setting.[4] In acquired resistance, mutations or genomic alterations develop owing to therapy selection pressures that favor continued cancer cell proliferation despite the loss of primary estrogen signaling. In primary resistance, which affects a smaller pool of patients, mutations or genomic alterations are present in the untreated breast cancer.[5] Examples of acquired genomic alterations leading to endocrine therapy resistance include mutations in the estrogen receptor alpha (*ESR1*) gene and mutations or alterations leading to an increased activation of growth factor pathways, such as phosphoinositide 3-kinase (PI3K). Primary genomic alterations include loss of p16, fibroblast

growth factor receptor (*FGFR*) gene amplifications, and *MYC* amplification or overexpression.[5-8]

In this review, we discuss the development of current and investigational targeted therapies for patients with advanced HR+ breast cancer.

Standard-of-Care Strategies to Address Endocrine Resistance

Genomic Alterations in HR+ Breast Cancer

Resistance to endocrine therapy can be related to a variety of genomic alterations in several different pathways (Figure). One of the challenges of addressing the genomic alterations in advanced HR+ breast cancer is the diversity of mutations that can be implicated in treatment resistance. Several breast cancer cell pathways can be altered to favor cancer progression and carcinogenesis, and many of the genomic alterations that are seen in advanced HR+ breast cancer occur in 10% or fewer of patients.[5,7]

The major steps in the ER pathway include estrogen binding to the ER with signaling through cyclin-dependent kinases 4 and 6 (CDK4/6) and cyclin D, leading to the phosphorylation of retinoblastoma protein (Rb). Phosphorylation of Rb releases the E2F transcription factor and triggers progression from G1 to S in the cell cycle, leading to cancer cell proliferation and tumor growth. Multiple pathways and proteins other than direct binding with the ER route through CDK4/6 and lead to Rb phosphorylation.. These include alterations such as inactivation of p16, which inhibits CDK4/6, a common finding in breast cancer[9]; mutations in *PIK3CA* and *TP53*, which are found in about 40% of patients with metastatic HR+ breast cancer; and alterations in proteins such as PTEN, ERBB2, MYC, and ARID1A, which occur in 10% or fewer patients but still drive cell proliferation.[5,7]

These alterations ultimately favor the development of endocrine therapy resistance (ie, continued cellular proliferation despite endocrine blockade). Some of the propensities to resistance are present in untreated primary HR+ breast cancer, such as genomic alterations in *PIK3CA*, *HER2*, *AKT*, and *FGFR*,[5,7] whereas others develop through selection pressure with endocrine treatment. Mutations in *ESR1* are one of the more common treatment-acquired alterations. *ESR1* mutations are present in only 3% of untreated HR+ breast cancers, but in 25% of AI-treated HR+ breast cancers.[7] Selective estrogen receptor downregulators (SERDs) such as fulvestrant were subsequently developed to address *ESR1* mutations.

CDK4/6 Inhibitors

Inactivation of p16 and modifications in the Rb/CDK4/CDK6/cyclin D pathway are present in approximately 50% of primary HR+ breast cancers, driving cancer cell growth and contributing to endocrine resistance.[8,10] The addition of specific inhibitors of CDK4/6 (palbociclib [Ibrance, Pfizer], ribociclib [Kisqali, Novartis], abemaciclib [Verzenio, Lilly], and dalpiciclib [SHR6390]) to an endocrine backbone (an AI or fulvestrant) prolongs endocrine sensitivity, and CDK4/6 inhibitors are now recommended as part of first-line therapy for patients with metastatic HR+ disease. Palbociclib, ribociclib, abemaciclib, and dalpiciclib all prolong progression-free survival (PFS) in the first- or second-line setting,[11-15] and an overall survival (OS) benefit has been shown in the first-line setting for premenopausal women,[12] although it has not yet been reported in postmenopausal women.[11,16,17] In the second-line setting, the combination of either fulvestrant and abemaciclib or fulvestrant and ribociclib prolonged OS in the intention-to-treat population and in patients with endocrine-resistant disease.[14,18] Although the combination of fulvestrant and palbociclib did not result in a significant OS difference in the intention-to-treat population, it did appear to increase OS in the patients with endocrine-sensitive breast cancer on subgroup analysis.[13]

Evidence suggests that patients with HR+ breast cancer that has progressed during endocrine therapy and CDK4/6 inhibitor treatment could still benefit from an endocrine-based regimen if the correct resistance mechanism were addressed. In a small study looking at patients treated with letrozole/palbociclib, new *ESR1* mutations developed over the course of treatment.[19] Another correlative study (PALOMA-3) looking at the cell-free circulating tumor DNA of patients treated with fulvestrant/palbociclib found that 30% of them had acquired new mutations in genes such as *ESR1*, *PI3KCA*, *ERBB2*, *FGFR,* and *Rb* over the course of treatment.[20] Interestingly, the mutational profile of patients treated with fulvestrant was the same as that of the patients treated with fulvestrant/palbociclib except for the presence of *Rb* mutations, which developed in 5% of patients after treatment with fulvestrant/palbociclib but not in those treated with fulvestrant alone. This finding suggests that treatment is pressuring both the development of mutations such as those in *ESR1* that will directly evade the endocrine backbone and alterations in other pathways that drive breast cancer proliferation, rather than that the breast cancer is developing independence from estrogen-based signaling. With continued tumor dependence on estrogen signaling, it is reasonable to posit that additional endocrine-based treatments could be effective if paired with the correct adjunct treatment to address the resistant pathway. This idea was supported by the results of the BOLERO-2 randomized phase 3 trial, which showed that the combination of everolimus, a mammalian target of rapamycin (mTOR) inhibitor, plus exemestane improved

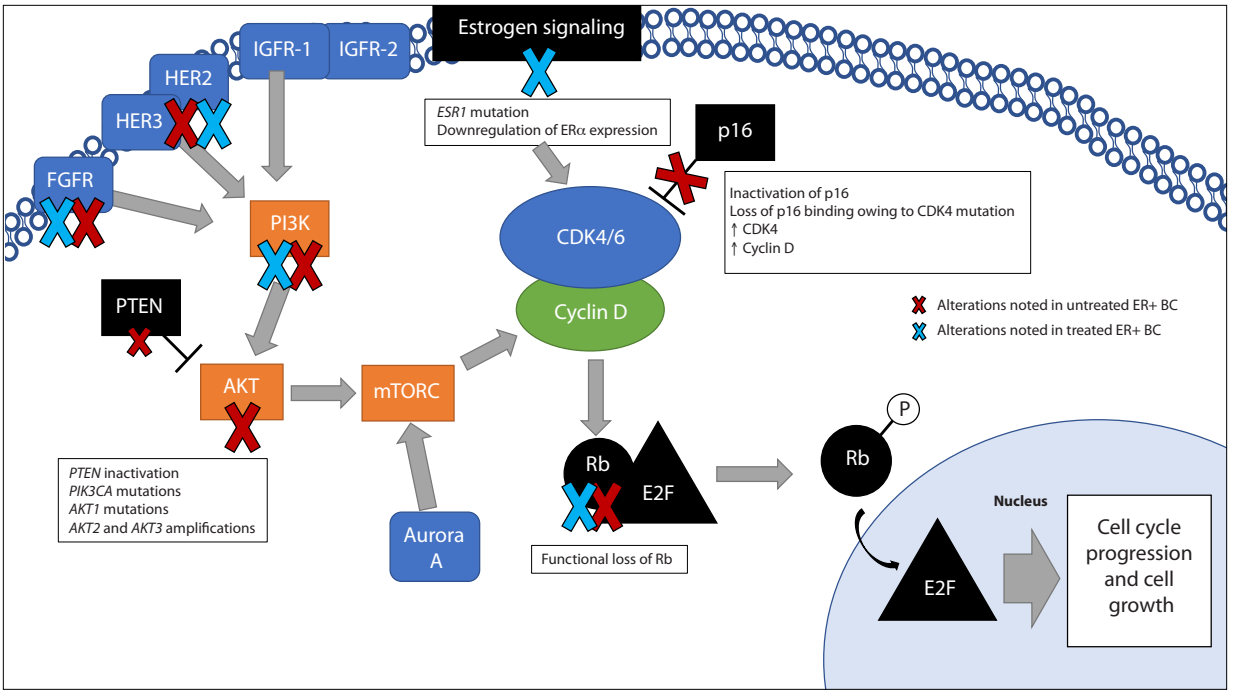

Figure. Estrogen receptor signaling in HR+ breast cancer.

AKT, RAC-alpha serine/threonine protein kinase; BC, breast cancer; CDK4/6, cyclin-dependent kinases 4 and 6; ER+, estrogen receptor–positive; ERα, estrogen receptor alpha; *ESR1,* estrogen receptor alpha gene; FGFR, fibroblast growth factor receptor; HER2, human epidermal growth factor receptor 2; IGFR-1, insulin-like growth factor receptor 1; mTORC, mammalian target of rapamycin complex; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; Rb, retinoblastoma protein.

PFS vs exemestane alone in advanced HR+ breast cancer that had already been exposed to AIs.[21]

PI3K Inhibitors

Because almost 40% of HR+ breast cancers have a mutation in *PIK3CA*,[22] researchers became interested in therapies targeting the PI3K pathway. PI3K has 4 isoforms: alpha, beta, delta, and gamma. In 2019, the alpha-specific PI3K inhibitor alpelisib (Piqray, Novartis) was combined with fulvestrant for the treatment of AI-resistant, *PIK3CA*-mutated advanced HR+ breast cancer and became the second PI3K pathway inhibitor approved by the Food and Drug Administration (FDA). Approval was based on the clinical and statistically significant PFS advantage seen in the SOLAR-1 trial,[23] albeit without a statistically significant OS benefit.[24] This was the first approval of a drug for advanced HR+ breast cancer that required companion genomic testing to identify patients who would benefit from the drug, and early genomic testing has since become a standard in advanced HR+ breast cancer. However, only 3% of patients in the pivotal trial had prior exposure to CDK4/6 inhibitors.[23] Nevertheless, the combination of fulvestrant and alpelisib appears to be a feasible second-line option for patients previously exposed to CDK4/6 inhibitors, as preliminary results of a nonrandomized phase 2 trial of fulvestrant/alpelisib or letrozole/alpelisib in patients with *PI3KCA*-mutated, HR+/HER2– cancer that previously progressed on endocrine therapy plus CDK4/6 inhibitors demonstrated clinical activity.[25]

Additional PI3K inhibitors are under development. Buparlisib and pictilisib are both pan-inhibitors of PI3K. GDC-0077, like alpelisib, is an alpha isoform–specific inhibitor; copanlisib (Aliqopa, Bayer) is alpha isoform– and delta isoform–specific; and taselisib is "beta-sparing" and targets the alpha, delta, and gamma isoforms. Buparlisib, pictilisib, and taselisib had modest PFS benefits in HR+ breast cancer, but frequent interruptions because of toxicity limited further development of these drugs.[26,27] Copanlisib is still being explored as a potential agent because of preliminary benefit, in combination with letrozole, in both *PIK3CA*- and *PTEN*-mutated HR+ breast cancer cell lines.[28] Two trials, one with copanlisib/fulvestrant (NCT03803761) and one with copanlisib/abemaciclib/fulvestrant (NCT03939897) in the post-CDK4/6 inhibitor setting (Table 1), are currently underway.

Novel Targeted Therapies for Patients With Endocrine-Resistant HR+ Breast Cancer

Most patients will move on to treatment with chemotherapy after their disease has progressed on the available endocrine therapies with or without approved targeted treatments. However, multiple functional genomic alterations potentially could be targeted for therapeutic benefit and reversal of endocrine therapy resistance. The next section reviews the various agents under investigation.

Novel Agents Targeting the Estrogen Receptor

Fulvestrant is an FDA-approved SERD that is administered via intramuscular injection. SERDs are antagonists of the ER, binding to it and causing degradation of the receptor protein. *ESR1* mutations result in cellular proliferation through both ER ligand–dependent and ER ligand–independent gene transcription, increased ER coactivator binding in the presence of tamoxifen, and decreased ER degradation in the presence of fulvestrant.[29]

Multiple SERDs under development that are orally administered appear to have reasonable safety profiles and to have activity against breast cancers with *ESR1* mutations. These include GDC 9545,[30,31] elacestrant,[32] AZD9496,[33] AZD9833,[34] SAR 439859,[35,36] LSZ102,[37] and rintodestrant.[38] Only AZD9496 has been compared directly with fulvestrant, and at the dose tested, it did demonstrate ER degradation, although the rates of degradation were not superior to those seen with fulvestrant.[33] Multiple ongoing trials are looking to combine novel SERDs with CDK4/6 or PI3K inhibitors, including NCT04059484, which is evaluating SAR439859 in combination with targeted therapies.

The complete estrogen receptor antagonist (CERAN) OP-1250, which is an oral small molecule, has demonstrated activity in preclinical models of HR+ breast cancer.[39] Like fulvestrant, OP-1250 differs from many of the other estrogen-binding agents in that it has no activity as agonist binding to uterine ER and therefore no increased risk for endometrial cancer.[39] NCT04505826 is a phase 1/2 study evaluating OP-1250 in advanced HR+ breast cancer.

Whereas SERDs and CERANs bind reversibly to the ER, the selective estrogen receptor covalent antagonist (SERCA) H3B-6545 binds covalently to the Cys530 residue of the ER. Like the novel SERDs, this is an oral agent. In heavily pretreated patients, H3B-6545 demonstrated activity in those with wild-type or *ESR1*-mutated HR+ breast cancers; the only notable side effect was bradycardia.[40] H3B-6545 is being studied in combination with palbociclib (NCT04288089).[41]

AKT Inhibitors

Mutations in *PIK3CA* and *AKT* are generally mutually exclusive.[42,43] PI3K phosphorylates RAC-alpha serine/threonine protein kinase (AKT), leading to downstream signaling that promotes cell growth. PTEN acts as an inhibitor of the PI3K pathway by dephosphorylating the same targets. *PIK3CA* mutations do not clearly result in an increase in AKT phosphorylation, regardless of PTEN status (high or low), but mutations in *AKT1* do seem to increase AKT phosphorylation, even in the presence of high PTEN levels.[44] The E17K mutation in *AKT1* is associated with HR+ and *HER2*-nonamplified breast cancers.[45] The duration of CDK4/6 inhibitor therapy is similar in patients with wild-type and those with mutant *AKT1*, but there is some suggestion that tumors with *AKT1* mutations benefit from mTOR inhibitors longer do than tumors with wild-type *AKT1*.[46]

Ipatasertib and capivasertib are 2 AKT inhibitors that are currently under investigation or have been tested in HR+ breast cancer. Ipatasertib was tolerable in combination with fulvestrant and palbociclib, with some evidence of clinical activity in the preliminary results of the phase 1b trial.[47] At this point, however, further development of ipatasertib in HR+ breast cancer has been discontinued. Capivasertib has demonstrated activity in patients with AKT^{E17K}-mutated HR+ breast cancer. In patients who received capivasertib monotherapy, an objective response rate (ORR) of 20% was seen, and an ORR of 36% with capivasertib/fulvestrant was noted in patients previously treated with fulvestrant.[48] In that study, PFS was improved in patients who had an early decrease in AKT^{E17K} mutations in plasma.

A randomized phase 2 study of fulvestrant/capivasertib in postmenopausal women with endocrine-resistant advanced HR+ breast cancer (not selected for *AKT* mutations) demonstrated better PFS in women treated with the combination than in women treated with fulvestrant alone.[49] This study did explore if PTEN/PI3K pathway alterations, specifically loss of *PTEN* or *PI3KCA* hotspot mutations in exon 9 or 20, had an effect on ORR or PFS in subgroup analyses. Patients without loss of *PTEN* or *PI3KCA* mutations had better PFS with capivasertib/fulvestrant than with fulvestrant alone, but for the subgroup of patients with loss of *PTEN* or *PI3KCA* mutations, the difference in PFS was not statistically significant. This suggests that mutations in *PTEN* and *PI3KCA* can unfortunately overcome AKT blockade with capivasertib. Nevertheless, the results of the phase 2 trial showing activity in patients with wild-type *PTEN* and *PI3KCA* remain promising, and phase 3 studies are ongoing for the combination of fulvestrant and capivasertib.

mTORC Inhibitors

Mammalian target of rapamycin complexes 1 (mTORC1) and 2 (mTORC2) are downstream of AKT but upstream of the CDK4/6 complex. The mTORC1 inhibitor everolimus was FDA-approved with tamoxifen, an AI,

Table 1. Clinical Trials That Are Exploring Targeted Therapy in Advanced Estrogen Receptor–Positive Breast Cancer

Investigational Agent Class	Clinical Trial No.	Investigational Agent(s), Phase	Biomarker of Interest
mTOR inhibitor*	NCT02599714	Vistusertib + palbociclib + fulvestrant, 2	
PI3K inhibitor	NCT01633060		*PIK3CA* mutation
	NCT01437566	Buparlisib + fulvestrant, 3	
	NCT02340221	Pictilisib + fulvestrant, 2	*PIK3CA* mutation
	NCT04191499	Taselisib + fulvestrant, 3	*PIK3CA* mutation
	NCT03939897	GDC-0077 + palbociclib + fulvestrant, 2/3	*PIK3CA*, *PTEN* alterations, pAKT levels
		Copanlisib + abemaciclib + fulvestrant, 1/2	
SERD/CERAN	NCT04214288	AZD-9833, 2	
	NCT04059484	SAR439859, 2	
	NCT04514159	Zn-c5 + abemaciclib, 1b	
	NCT03560531	Zn-c5 + palbociclib, 1/2	
	NCT04505826	OP-1250, 1/2	
AKT inhibitor	NCT03337724	Ipatasertib + paclitaxel, 2/3	*PIK3CA*, *PTEN*, or *AKT1* altered
	NCT04060862	Ipatasertib + palbociclib + fulvestrant, 3	
	NCT04305496	Capivasertib + fulvestrant, 3	
FGFR inhibitor	NCT03238196	Erdafitinib + palbociclib + fulvestrant, 1	*FGFR* amplification
HER2-directed	NCT04494425	Trastuzumab deruxtecan, 3	HER2-low by IHC or ISH
	NCT01670877	Neratinib + fulvestrant + trastuzumab, 2	*HER2*-nonamplified with *HER2* mutation
IGFR-1 inhibitor	NCT03659136	Xentuzumab + everolimus + exemestane, 2	
TKI	NCT01441947	Cabozantinib + fulvestrant, 2	
	NCT03854903	Bosutinib + palbociclib + fulvestrant, 2	
Aurora A kinase inhibitor	NCT02860000	Alisertib + fulvestrant, 2	
SARM	NCT04142060	Enzalutamide (+ exemestane), 2	HER2-enriched by PAM50 profile
	NCT02463032	Enobosarm, 2	
Bcl-2 inhibitor	NCT03900884	Venetoclax + palbociclib + letrozole, 1	
	NCT03584009	Venetoclax + fulvestrant, 2	
BET inhibitor	NCT02964507	GSK525762 + fulvestrant, 2	
HDAC inhibitor	NCT02115282	Entinostat + exemestane, 3	
DNMT inhibitor	NCT04134884	ASTX727 + talazoparib	*BRCA1/2* wild-type

* Combinations for which further clinical development in ER+ breast cancer has been halted are in red type.

AKT, RAC-alpha serine/threonine protein kinase; Bcl-2, B-cell lymphoma 2; BET, bromodomain and extraterminal; BRCA1/2, breast cancer type 1 and type 2 susceptibility gene; CERAN, complete estrogen receptor antagonist; DNMT, DNA methyltransferase inhibitor; ER+, estrogen receptor–positive; FGFR, fibroblast growth factor receptor; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; IGFR-1, insulin-like growth factor receptor 1; pAKT, phosphorylated RAC-alpha serine/threonine protein kinase; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; SARM, selective androgen receptor modulator; SERD, selective estrogen receptor downregulator; TKI, tyrosine kinase inhibitor.

or fulvestrant on the basis of a significant improvement in PFS, particularly in patients with acquired endocrine therapy resistance, although no effect on OS was seen.[21,50,51] The encouraging results indicating that everolimus could address endocrine resistance led to interest in dual mTORC1 and mTORC2 inhibition. Vistusertib is a dual mTORC1 and mTORC2 inhibitor that in combination with fulvestrant did not improve PFS vs fulvestrant alone, and the vistusertib/fulvestrant combination was inferior to fulvestrant/everolimus.[52] However, in preclinical studies, the addition of CDK4/6 inhibition to fulvestrant/vistusertib increased the effectiveness of the combination in inducing cell growth arrest and also delayed the development of treatment resistance.[53] The combination of everolimus/exemestane/ribociclib showed promising activity in patients who had previously received CDK4/6 inhibitors, with 41% of patients deriving clinical benefit.[54]

Receptor Tyrosine Kinase Inhibitors

Multiple tyrosine kinase pathways upstream from PI3K and mTORC can drive breast cancer cell proliferation as well as endocrine resistance. Current tyrosine kinase receptors of therapeutic interest in endocrine-resistant HR+ breast cancer include fibroblast growth factor receptor 1 (FGFR1), human epidermal growth factor receptor 2 (HER2), and insulin-like growth factor receptor 1 (IGFR-1). Signaling through these pathways may contribute to the development of resistance during treatment with combination endocrine blockade and CDK4/6 inhibition, and several clinical trials are exploring triplet drug combinations in patients whose disease has progressed through CDK4/6 inhibitor treatment.

Amplification and activating mutations of *FGFR1* are associated with a higher frequency of resistance to endocrine-based therapies in patients with early-stage HR+ breast cancer.[55,56] These alterations can be primary or acquired through treatment.[7,20] In patient-derived xenograft mouse models of *FGFR1*-amplified HR+ breast cancer, the combination of fulvestrant, palbociclib, and erdafitinib (Balversa, Janssen), a pan-FGFR inhibitor, exhibited more anti-growth activity and suppression of Ki-67 than did fulvestrant/palbociclib alone.[56] A clinical trial of fulvestrant/palbociclib/erdafitinib is currently ongoing in patients with metastatic HR+ breast cancer that is refractory to endocrine therapy and CDK4/6 inhibitors, and preliminary results show clinical activity in patients with high levels of *FGFR1* amplifications.[57]

Mutations in *ERBB2* and *ERBB3* have also been associated with endocrine resistance. In a study performing whole-exome sequencing in patients with HR+ metastatic breast cancer, approximately 7% of patients had activating *ERBB2* mutations in biopsy specimens from metastatic sites; some of these alterations were likely acquired through treatment selection pressure.[58] Another study found that these mutations are slightly more common in invasive lobular carcinomas than in ductal carcinomas.[59]

Preclinical studies have shown that fulvestrant increases HER2 and EGFR phosphorylation in HR+ and *HER2*-amplified breast cancers, and that treatment with neratinib (Nerlynx, Puma Biotechnology) in these breast cancers induces ER transcriptional activity.[60] Currently, 2 trials are looking at the use of HER2-targeted therapies in *HER2*-nonamplified or HER2-low (0+ or 1+ by immunohistochemistry) breast cancers. One study (NCT01670877) is evaluating neratinib in combination with fulvestrant for metastatic HR+ breast cancer with *ERBB2* mutations. An interim analysis demonstrated activity, with a 33% ORR in the cohort, a 17% ORR in patients previously treated with fulvestrant, and a 26% ORR in patients previously treated with CDK4/6 inhibitors.[61] A retrospective genomic analysis in a subset of the patients enrolled in SUMMIT suggested that those with multiple mutations in *HER2* or *HER3* at baseline had worse outcomes with neratinib, and that the development of additional *HER2*-activating mutations was a potential mechanism of acquired resistance to neratinib.[62] In *HER2*-amplified breast cancers, the combination of trastuzumab/neratinib prevented reactivation of HER3 and AKT, which is thought to be a mechanism of neratinib resistance.[63] The SUMMIT trial was amended to add trastuzumab at the time of progression on fulvestrant/neratinib to target HER2 with multiple agents. A second phase 3 trial targeting the HER2 pathway is evaluating the antibody-drug conjugate trastuzumab deruxtecan (Enhertu, Daiichi-Sankyo/AstraZeneca) vs standard-of-care chemotherapy in metastatic HER2-low breast cancer. In the phase 1b study, the ORR was 37%, with a 10.4-month duration of response in heavily pretreated patients,[64] which was encouraging. Final results from both of these studies are pending.

Finally, the IGF-1 and IGF-2 receptors may play a role in endocrine resistance through upregulation of IGF signaling.[65] Several clinical trials have examined various agents targeting the IGF pathway, but thus far none have shown benefit.[66] Xentuzumab is a humanized neutralizing antibody against IGF-1 and IGF-2. In a phase 1b/2 study of xentuzumab in combination with exemestane and everolimus, no PFS benefit was observed with xentuzumab vs xentuzumab/everolimus/exemestane.[67] However, subgroup analysis indicated a possible benefit in patients without visceral metastases, and a second clinical trial (NCT03659136) in this patient population is underway.

Protein Kinase Inhibitors

Both cabozantinib (Cabometyx, Exelixis) and bosutinib (Bosulif, Pfizer) are protein kinase inhibitors that are currently under investigation in metastatic HR+ breast cancer. Cabozantinib, which inhibits MET and vascular endothelial growth factor receptor 2 (VEGFR2), has shown activity as monotherapy in patients with HR+ breast cancer.[68] Bosutinib, a dual SRC/Abl inhibitor, has been studied in combination with letrozole and in combination with exemestane in the early treatment of metastatic HR+ breast cancer. Several patients responded to either cabozantinib or bosutinib in both studies, but both studies were discontinued owing to drug-related liver toxicity.[69,70] Several other trials are currently in progress that pair cabozantinib with fulvestrant and bosutinib with fulvestrant and palbociclib.

Aurora A kinase is a serine/threonine kinase that controls the segregation of DNA during mitosis; at high levels of overexpression, it is a negative prognostic marker in HR+/HER2– breast cancer.[71] Aurora A kinase has been found to contribute to endocrine resistance by driving

downregulation of ERα through SMAD5.[72] In a phase 1 study combining fulvestrant with alisertib, an aurora A kinase inhibitor, in patients with endocrine-resistant HR+ breast cancer, the majority of patients derived clinical benefit during the treatment, with a median PFS of 12.4 months.[73] A randomized phase 2 study of alisertib with and without fulvestrant demonstrated that fulvestrant did not significantly augment alisertib activity (ORR, 20%), but the study did affirm that alisertib has activity as a monotherapy, achieving a 17.8% ORR in a group of patients who had previously been treated with fulvestrant, AIs, and CDK4/6 inhibitors.[74]

Apoptosis Pathway

Bcl-2 is an apoptosis regulator that is expressed in most HR+ breast cancers, and when it was inhibited in preclinical mouse models, the antitumor efficacy of tamoxifen was improved.[75] In a phase 1 study looking at the combination of tamoxifen and venetoclax (Venclexta, AbbVie), a Bcl-2 inhibitor, the most common side effects were nausea, infection, fatigue, and rash, with no patients requiring study discontinuation owing to toxicity.[76] Venetoclax and tamoxifen demonstrated clinical activity, particularly at the highest dose level, with clinical benefit seen in patients whose disease had previously progressed on tamoxifen as well as first-line treatment with a CDK4/6 inhibitor and an AI. In a phase 2 study, the combination of fulvestrant and venetoclax did not significantly improve PFS or OS in comparison with fulvestrant alone.[77] An ongoing trial is evaluating venetoclax in combination with letrozole/palbociclib.[78]

Androgen Receptor Targeting

The androgen receptor (AR) is expressed in more than 80% of early HR+ breast cancers in postmenopausal women[79] and may contribute to endocrine resistance.[80] A randomized phase 2 study evaluating a combination of the AR inhibitor enzalutamide (Xtandi, Astellas) and exemestane in advanced HR+ breast cancer found no PFS benefit in the overall cohort, but it did note that patients with high levels of circulating AR mRNA seemed to benefit.[81] A current clinical trial using enzalutamide is focusing on patients who have HR+ breast cancer with the HER2-enriched PAM 50 phenotype (NCT04142060). Approximately half of the breast cancers in the HER2-enriched subgroup are not *HER2*-amplified, and in these breast cancers, AR may be a driver of breast cancer proliferation.[82] Other AR antagonists studied in breast cancer include bicalutamide, which did not show any benefit in combination with AIs,[83] and orteronel.[84]

Androgen agonists are also of interest in advanced HR+ breast cancer. The AR can function as a tumor suppressor in HR+ breast cancer, and preclinical studies have demonstrated that androgen agonists can induce antineoplastic activity in endocrine-resistant and CDK4/6 inhibitor–resistant breast cancer.[85] A phase 2 trial evaluating enobosarm, a selective AR agonist, in advanced HR+ breast cancer demonstrated a clinical benefit in about one-quarter of heavily pretreated patients.[86] Further analysis of the results based on tumor AR staining showed that breast cancers with at least 40% AR staining had an ORR of 48%, whereas breast cancers with less than 40% staining had an ORR of 0%.[87] On the basis of these results, enobosarm is moving forward into a phase 3 trial (NCT04869943) in AR+/ER+/HER2– advanced breast cancer.

Epigenetic Drugs

Three groups of epigenome-modulating drugs are under investigation for treating HR+ breast cancer: histone deacetylase (HDAC) inhibitors, bromodomain and extraterminal domain (BET) protein inhibitors, and DNA methyltransferase (DNMT) inhibitors. In vitro, HDAC inhibitors reverse the transcriptional upregulation of c-MYC and Bcl-2 in tamoxifen-resistant breast cancer cells and restore endocrine sensitivity.[88] A randomized phase 2 study (ENCORE 301) that looked at the combination of entinostat, an HDAC inhibitor, with exemestane in postmenopausal women with advanced HR+ breast cancer suggested that the addition of entinostat to exemestane significantly prolonged PFS.[89] However, the subsequent phase 3 study did not show a PFS or OS benefit.[90]

Similarly, preclinical studies of BET inhibitors in combination with fulvestrant have demonstrated activity in endocrine therapy–resistant breast cancer,[91] as well as in reversing resistance to everolimus.[92] Results from a phase 1/2 study combining the pan-BET inhibitor GSK525762 with fulvestrant in patients with advanced ER+ breast cancer are currently pending.[93]

DNMT inhibitors are being studied in combination with poly(ADP)-ribose polymerase (PARP) inhibitors in *BRCA* wild-type cancers. In patients with germline *BRCA*-mutated breast cancer, who account for approximately 10% of patients with HER2-negative metastatic breast cancer,[94] olaparib (Lynparza, AstraZeneca) improved PFS vs physician's choice of chemotherapy,[95] and although no OS benefit was observed in the overall cohort, patients with no prior chemotherapy seemed to derive some survival benefit.[96] Preclinical studies in breast cancer cell lines have suggested that using a DNMT inhibitor concurrently with a PARP inhibitor enhances the antitumor effect of PARP inhibitors, irrespective of *BRCA* status.[97,98] This would expand the utility of PARP inhibitors to all patients, regardless of *BRCA* status. An ongoing clinical trial that is combining talazoparib (Talzenna, Pfizer) with ASTX727, a DNMT inhibitor,

Table 2. Phase 2/3 Trials of Immunotherapy Agents in Combination With Endocrine Therapy and/or Targeted Treatments in Advanced Estrogen Receptor–Positive Breast Cancer

Investigational Agent Class	Clinical Trial Number	Investigational Agent(s), Phase	Biomarker of Interest
Endocrine therapy	NCT03874325 NCT03280563	Durvalumab + AI, 2 Atezolizumab + fulvestrant, 1b/2	
CDK4/6 inhibitor	NCT03294694 NCT03280563 NCT02778685	Spartalizumab + ribociclib + fulvestrant, 1b Atezolizumab + abemaciclib + fulvestrant, 1b/2 Pembrolizumab + palbociclib + letrozole, 2	
VEGF-A inhibitor	NCT03280563	Bevacizumab + atezolizumab + exemestane, 1b/2 Bevacizumab + atezolizumab + fulvestrant, 1b/2	
AKT inhibitor	NCT03280563	Ipatasertib + atezolizumab, 1b/2 Ipatasertib + atezolizumab + fulvestrant, 1b/2	
HDAC inhibitor	NCT03280563	Entinostat + atezolizumab, 1b/2	
TGF-β1 receptor inhibitor	NCT03685591	PF-06952229 + palbociclib + letrozole, 1	High TGF-β signature
CXCR4 agonist	NCT03786094	Balixafortide + eribulin, 3	CXCR4 expression by IHC

AKT, RAC-alpha serine/threonine protein kinase; CDK4/6, cyclin-dependent kinases 4 and 6; CXCR4, C-X-C chemokine receptor type 4; ER+, estrogen receptor–positive; HDAC, histone deacetylase; IHC, immunohistochemistry; TGF-β1, transforming growth factor beta 1; VEGF-A, vascular endothelial growth factor A.

includes patients who have endocrine therapy–resistant HR+ breast cancer.

Immunotherapy in HR+ Breast Cancer

Immunotherapy is appealing because of its ability to induce durable responses in other tumor types that can persist even while the patient is off therapy. In triple-negative breast cancer (TNBC), for which 2 first-line immunotherapy-based regimens are currently approved by the FDA, the combination of chemotherapy and immunotherapy has demonstrated clinical benefit in the first-line setting.[99,100] However, the combination of chemotherapy and immunotherapy has not yet been successful in advanced HR+ breast cancer. The rates of immune infiltration, defined by tumor-infiltrating lymphocytes,[101] are lower in HR+ breast cancer, and the ORR in programmed death ligand 1 (PD-L1)–positive HR+ breast cancer when treated with programmed death 1 (PD-1) monotherapy is low (12%).[102] A trial exploring eribulin (Halaven, Eisai) in combination with pembrolizumab (Keytruda, Merck) did not did not find any increase PFS or OS in patients with HR+ cancers, and approximately half of the patients experienced grade 3/4 toxicities.[103] Nonetheless, other clinical trials will use other chemotherapy agents in combination with immunotherapy, including weekly paclitaxel with pembrolizumab in luminal-B HR+ breast cancer (NCT03841747) and nab-paclitaxel (Abraxane, Bristol Myers Squibb) in combination with nivolumab (Opdivo, Bristol Myers Squibb) with or without ipilimumab (Yervoy, Bristol Myers Squibb; NCT04132817).

The combination of immunotherapy and endocrine treatments may have some efficacy in advanced HR+ breast cancer because endocrine-based therapies may modulate the tumor microenvironment. In preclinical studies, fulvestrant decreased the infiltration of neutrophils and macrophages and also decreased the levels of cytokine receptors and chemokines associated with tumor progression.[104] The combination of endocrine therapy and immune checkpoint inhibitors could be synergistic by promoting antitumor effects through both innate and adaptive immunity. Multiple trials are currently ongoing to evaluate the combination of PD-1 and PD-L1 inhibitors, endocrine therapy, and targeted therapies for the treatment of HR+ breast cancer (Table 2) .

The addition of targeted agents such as HDAC inhibitors and AKT inhibitors may also enhance the immunotherapy effect. Preclinical studies in HR+ breast cancer cell lines show that AKT inhibitors can block estradiol-induced upregulation of PD-L1 expression.[105] However, although HDAC inhibitors do seem to enhance PD-L1 and human leukocyte antigen – DR isotype (HLA-DR) expression in triple-negative breast cancer cell lines,[106] this effect was not observed in HR+ breast cancer lines. This lack of effect was confirmed in the clinical setting. A trial that combined vorinostat (Zolinza, Merck), an HDAC inhibitor, with pembrolizumab and tamoxifen in patients with advanced HR+ breast cancer showed that baseline T-cell exhaustion and a decrease in regulatory

T cells with treatment were associated with an objective response to combination therapy.[107] However, the ORR in the overall cohort was low (4%), and it was observed that neither vorinostat nor pembrolizumab seemed to induce a positive shift in the tumor microenvironment in terms of promoting an immune response.[107]

Cytokine and chemokine signaling that creates a protumor stromal tumor microenvironment may also be a reasonable target to consider in HR+ breast cancer with or without the addition of immune checkpoint inhibitors. C-X-C chemokine receptor type 4 (CXCR4) signaling contributes to immunosuppression through fibroblast recruitment, which results in fibrosis, angiogenesis, and a hypoxic tumor microenvironment.[108] The combination of balixafortide, a CXCR4 inhibitor, and eribulin showed reasonable safety in heavily pretreated patients with advanced HR+ breast cancer, achieving an ORR of 30%.[109] Results from a phase 3 trial are awaited.

Conclusions

Endocrine-based therapies for HR+ breast cancer are the preferred initial treatment strategy owing to durable responses in the majority of patients, favorable toxicity profiles, and relatively convenient administration. Endocrine resistance develops through multiple potential mechanisms, and translational studies looking at CDK4/6-resistant breast cancers suggest that endocrine therapy could still be effective if a druggable genomic alteration were identified. Despite the current challenges in the field, the success of CDK4/6 and PI3K inhibitors in combination with either an AI or a SERD illustrates the promise of targeted therapies in advanced HR+ breast cancer. Continued investigation into the mechanisms and drivers of endocrine resistance, in addition to the development of new, more specific targeted agents, is needed to extend the endocrine-based options for HR+ breast cancer therapy.

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