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Review

Evolving perspectives on the treatment of HR+/HER2+ metastatic breast cancer

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***Abstract*:** Breast cancer (BC) with expression of the estrogen receptor (ER) and/or progesterone receptor (PR) protein and with overexpression/amplification of the human epidermal growth factor receptor 2 (HER2), termed hormone receptor-positive (HR+)/HER2+ BC, represents ~10% of all BCs in the United States. HR+/HER2+ BC includes HER2+ BCs that are ER+, PR+, or both ER+ and PR+ (triple-positive BC). Although the current guideline-recommended treatment combination of anti-HER2 monoclonal antibodies plus chemotherapy is an effective first-line therapy for many patients with HER2+ advanced disease, intratumoral heterogeneity within the HR+/HER2+ subtype and differences between the HR+/HER2+ subtype and the HR–/HER2+ subtype suggest that other targeted combinations could be investigated in randomized clinical trials for patients with HR+/HER2+ BC. In addition, published data indicate that crosstalk between HRs and HER2 can lead to treatment resistance. Dual HR and HER2 pathway targeting has been shown to be a rational approach to effective and well-tolerated therapy for patients with tumors driven by HER2 and HR, as it may prevent development of resistance by blocking receptor pathway crosstalk. However, clinical trial data for such approaches are limited. Treatments to attenuate other signaling pathways involved in receptor crosstalk are also under investigation for inclusion in dual receptor targeting regimens. These include cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors, based on the rationale that association of CDK4/6 with cyclin D1 may play a role in resistance to HER2-directed therapies, and others such as phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway inhibitors. Herein, we will review the scientific and clinical rationale for combined receptor blockade targeting HER2 and ER for patients with advanced-stage HR+/HER2+ disease.

Keywords: CDK4/6 inhibitors, HR+/HER2+ breast cancer, metastatic breast cancer, resistance, triple-positive breast cancer

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Introduction

Breast cancer (BC) is a heterogeneous disease that is broadly classified into five major common clinical subtypes based on estrogen receptor (ER) expression, progesterone receptor (PR) expression, and human epidermal growth factor receptor 2 (HER2) expression or gene amplification. These five subtypes are the following: (a) hormone receptor-positive (HR+; i.e. tumors expressing ER, PR, or both)/HER2-negative (HER2−) disease; (b) HR+/HER2+ disease (which includes ER+/PR−/HER2+, ER−/PR+/HER2+, and triple-positive [ER+/PR+/HER2+] disease); (c) hormone receptor-negative (HR−)/HER2+ disease; (d) triple-negative (ER−/PR−/HER2−) disease; and (e) HER2-low, defined as immunohistochemical detection of HER2 protein at a 1+ or 2+ level in tumor cells, and lacking amplification of the gene encoding HER2, encompassing both HR+ and HR− patients.[1,2] Tumors classified as HR+/HER2−, which generally includes those that are HR+/HER2-low,[3] are

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the most common subtype, accounting for ~74% of all BCs.[4–6]

ER and PR are nuclear transcription factors that, in their canonical genomic signaling mode, dimerize following ligand binding in the cell cytoplasm and translocate to the nucleus, where they interact with steroid receptor co-activator and repressor molecules and other nuclear transcription factors on response elements (specific DNA sequences regulating gene transcription), resulting in the promotion of cell division, proliferation, and invasion.[7] It has become apparent that ER variants can also be associated with plasma membrane caveolae/lipid rafts where it can activate non-nuclear signaling, which is also termed rapid, nongenomic, or membrane initiated steroid signaling in a variety of cell types.[8] HER2 is a cell surface receptor tyrosine kinase that stimulates multiple intracellular signaling pathways that support cell proliferation, cell migration/invasion, tumorigenesis, and survival;[9–11] thus, HER2+ status, characteristic of ~15% of BCs in the United States, is associated with clinical aggressiveness if not treated with anti-HER2 therapy.[4]

HR+/HER2+ tumors account for ~10% of all BCs in the United States[4–6] and, compared with HR+/HER2− tumors, are more likely to be high grade and are more prevalent in younger patients.[4] However, an analysis of a large sample of US women with newly diagnosed stage IV BC ($N = 14{,}000$) found that, likely owing to the robust efficacy of targeted anti-HER2 therapies, these aggressive HR+/HER2+ tumors are now actually associated with a lower risk of mortality than HR+/HER2− tumors.[12] A retrospective, population-based study of patients with HER2+ disease (in which information on endocrine therapy [ET] and HER2 therapy was not available) found that among all HER2+ tumors, HR+/HER2+ tumors are more likely than HR−/HER2+ tumors to be of lower grade, smaller size, and have less nodal involvement, and are less likely to be de novo stage IV.[13] Thus, the presence of HR+/HER2+ tumors is associated with superior overall patient survival compared with the presence of HR−/HER2+ tumors.[13] Within the HR+/HER2+ subtype, tumor behavior varies widely based on both patient-to-patient differences, as well as intratumoral heterogeneity, including the presence of other mutations, amplifications, and relative levels of HER2 overexpression along with variations in ER and/or PR expression.[13–15]

HR status is often not a primary consideration in treatment guidelines for HER2+ metastatic BC (mBC).[16–18] Current guidelines from the American Society of Clinical Oncology (ASCO®), the European Society for Medical Oncology (ESMO), and the National Comprehensive Cancer Network® (NCCN®) recommend anti-HER2 monoclonal antibody therapies (i.e. trastuzumab plus pertuzumab) in combination with taxane chemotherapy as standard of care for first-line systemic therapy for HER2+ mBC.[16–19] Results of prespecified subgroup analyses of the phase 3 CLEOPATRA (NCT00567190) and PERUSE trials (NCT01572038) in women with HER2+ locally recurrent or metastatic BC demonstrated the efficacy of this combination in both the HR+ and HR− subgroups.[20,21] The antibody-drug conjugate (ADC) trastuzumab emtansine (T-DM1), which contains trastuzumab linked to a microtubule inhibitor, is a single agent previously preferred as second-line therapy for patients with HER2+ mBC who had received prior treatment with trastuzumab and a taxane.[16,17,22] T-DM1 was effective in both the HR+ and HR− subgroups in the phase 3 EMILIA trial (NCT00829166).[23] However, the ADC trastuzumab deruxtecan, which contains trastuzumab linked to a topoisomerase I inhibitor, is now the preferred second-line treatment in patients with HER2+ mBC who previously received anti-HER2-based therapy.[18,19,24] This recommendation is based on an interim analysis of the head-to-head phase 3 DESTINY-Breast03 trial (NCT03529110), which showed that trastuzumab deruxtecan provided significantly longer progression-free survival (PFS) than T-DM1 regardless of HR status.[25] Trastuzumab deruxtecan also showed antitumor activity against T-DM1-pretreated HER2+ mBC, irrespective of HR status in the phase 2 DESTINY-Breast01 trial (NCT03248492).[26] Finally, a recent phase 3 trial (DESTINY-Breast04, NCT03734029) involving patients with HER2-low mBC showed that trastuzumab deruxtecan resulted in significantly longer PFS and overall survival (OS) than physician's choice of chemotherapy, regardless of HR status.[27] These results portend a new treatment approach going forward for significant numbers of patients previously categorized as having HER2− BC, but who now may be recategorized as HER2-low.

Although treatment guidelines for HER2+ mBC mention ET, the level of supporting evidence is low based on the lack of well-controlled phase 3 trials.[16–18] Thus, ET tends to be reserved for patients who do not tolerate chemotherapy or for post-chemotherapy maintenance, particularly in the first line with trastuzumab and pertuzumab.[14,18,21] Approved maintenance ET following chemotherapy discontinuation in PERUSE was used by 21% of patients,[21] and 23% of patients in CLEOPATRA who discontinued study treatment received maintenance ET.[20]

As described above, the substantial heterogeneity of HR+/HER2+ mBCs and their distinct molecular characteristics (e.g. variations in levels of ER and PR expression and HER2 overexpression, presence of other mutations) present both clinical challenges and opportunities regarding treatment optimization.[15] While HER2+ mBC remains for the most part an incurable disease (rare "exceptional responders" with durable complete clinical responses notwithstanding), there remains an unmet need for more tailored treatment approaches to maximize patient outcomes in HR+/HER2+ mBC, and to prevent overtreatment in some patients.[14] Resistance to treatment may develop through a variety of mechanisms, including crosstalk between treatment-attenuated HER2 signaling (kinase inhibitor- or antibody-based) and uninhibited HRs,[28] as well as between inhibited HRs and uninhibited HER2 signaling.[29] NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) currently recommend continued suppression of HER2 pathways after disease progression on anti-HER2 therapy.[19] A new area of interest in HR+/HER2+ mBC treatment is the addition of other inhibitors of these signaling pathways in the form of novel combination regimens. For instance, as association of cyclin-dependent kinase 4 and 6 (CDK4/6) with cyclin D1 appears to have a role in resistance to HER2-directed therapies, studies are now investigating the safety and efficacy of adding CDK4/6 inhibitors to combination regimens directed against HER2.[30] The aim of this review is to explore clinical and preclinical data supporting the use of tailored treatment regimens for individuals with HR+/HER2+ mBC.

Rationale for dual HR and HER2 pathway targeting in HR+/HER2+ mBC treatment

It is hypothesized that the heterogeneity of HR+/HER2+ mBC is such that the main oncogenic driver will vary among patients, with subsets of patients showing differential sensitivity to HER2 pathway targeting and HR pathway targeting, in turn, supporting the use of dual pathway targeting for optimal clinical benefit.[31] Further, evidence from *in vitro* and preclinical studies suggests that targeted blockade of HER2 alone may lead to HR activation *via* complex interactions and crosstalk between HR and HER2 signaling pathways, providing tumor cells with an escape route that results in anti-HER2 therapy resistance.[28,32–34] HR+/HER2+ tumors may therefore initially respond to HER2-targeted therapies but develop resistance over time.

Indeed, evidence from *in vitro* and preclinical studies found that ER-dependent genes were differentially upregulated in ER+/HER2+ BC cells resistant to lapatinib, a small molecule anti-epidermal growth factor receptor (EGFR)/HER2 tyrosine kinase inhibitor (TKI), implicating ER signaling in acquired lapatinib resistance.[32] A combination of lapatinib and anti-estrogen therapy prevented the development of acquired lapatinib resistance in ER+/HER2+ BC cells, providing a rationale for dual pathway targeting with ET and anti-HER2 therapies.[32,33] One of these studies further demonstrated that ERs became the primary drivers of tumor cell survival and proliferation in ER+/HER2+ BC cells with acquired resistance to trastuzumab and lapatinib.[33] In mouse xenograft models bearing ER+/HER2+ BC tumors treated with lapatinib alone or in combination with trastuzumab, anti-HER2 resistance was associated with increased ER expression/activity.[28] In another study, in the presence of HER2, ER activation stimulated the activity of EGFR, HER2, and other growth factor receptors, activating kinase cascades implicated in resistance, such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (AKT), leading to cell migration and upregulation of the chemokine receptor CXCR4.[34]

This HR/HER2 crosstalk-mediated resistance is bidirectional, as targeted blockade of HR alone also leads to changes in HER2 signaling.[35] Evidence from *in vitro* and preclinical studies suggests that HER2 overexpression can promote resistance to ET. For example, BC cell lines with acquired resistance to the ER antagonist tamoxifen showed increased mRNA and protein levels of HER2 and EGFR and increased HER2 and EGFR activity *via* phosphorylation; cell

growth was also inhibited by treatment with trastuzumab or the anti-EGFR TKI gefitinib, further implicating HER2 and EGFR signaling in acquired tamoxifen resistance.[29] In mouse ER+/HER2+ BC xenograft models, acquired ET resistance was associated with activation of HER2 and MAPK and was delayed with concomitant gefitinib treatment.[36,37]

Clinical data also support the involvement of HR/HER2 crosstalk in treatment resistance. Results of a secondary analysis of the phase 3 HERA trial (NCT00045032) in women with early-stage HER2+ BC who had undergone at least four rounds of adjuvant chemotherapy suggested that a subgroup of HER2+ tumors that express ER (measured by lower fluorescent *in situ* hybridization ratios or highest estrogen receptor 1 [*ESR1*] expression) may be less responsive to subsequent adjuvant trastuzumab therapy than those without ER expression, implying a relationship between ER signaling and resistance to trastuzumab.[38] In addition, in a retrospective analysis of patients with advanced HER2+ BC, those who had tumors with high ER expression (⩾30% of cells) had a reduced probability of response to trastuzumab plus chemotherapy (multivariate odds ratio 0.422; $p=0.009$) compared with patients who had tumors without high ER expression.[39]

An analysis of tumor biopsies from patients with HER+ BC who had received neoadjuvant treatment with lapatinib found that some HER2+ tumors that were initially ER− by diagnostic criteria became ER+ after treatment.[28] In the phase 3 ExteNET trial (NCT00878709) in early HER2+ BC, administration of neratinib, a TKI with activity against EGFR, HER2, and HER4, to achieve continuous HER blockade after adjuvant trastuzumab-based treatment improved disease-free survival in the subset of women with ER+/HER2+ BC when administered in combination with ET.[40] Notably, this benefit of neratinib was not observed in women with HR−/HER2+ BC.[40] Therefore, ER signaling in ER+/HER2+ tumors may be involved in the development of acquired resistance to anti-HER2 therapies.

Based on this crosstalk between HR and HER2 signaling pathways, combined HR and HER2 blockade should be considered in the treatment of HR+/HER2+ BC. Clinical trials that have investigated combined HR and HER2 blockade in postmenopausal women with HR+/HER2+ BC are outlined in Table 1. The phase 3 TAnDEM (NCT03517540) and eLEcTRA (NCT05386108) trials evaluated the combination of trastuzumab and an aromatase inhibitor (AI) as first-line therapy.[41,42] Another phase 3 trial evaluated the combination of lapatinib and letrozole as first-line therapy.[43] The phase 3 ALTERNATIVE trial (NCT01160211) assessed dual HER2 blockade with lapatinib and trastuzumab plus ER blockade with an AI in patients who had received prior trastuzumab and ET,[44] and the phase 2 PERTAIN trial (NCT01491737) assessed dual HER2 blockade with pertuzumab plus trastuzumab plus ER blockade with an AI in patients with no prior systemic nonhormonal anticancer therapy in the advanced setting.[45] Recently, in the open-label, noninferiority, phase 3, randomized controlled SYSUCC-002 trial (NCT01950182) performed at nine hospitals in China, 392 patients were randomly assigned to receive trastuzumab plus ET or trastuzumab plus chemotherapy as first-line treatment for HER2+ mBC.[46] After a median follow-up of 30.2 months, the median PFS was 19.2 months in the ET group and 14.8 months in the chemotherapy group (hazard ratio, 0.88; $p_{\text{noninferiority}} <0.0001$). Moreover, a significantly higher prevalence of toxicity was observed in the chemotherapy group compared with the ET group. Further, in a nonrandomized "real-world" analysis of National Cancer Database patients with HR+/HER2+ mBC who were treated between 2010 and 2015, among 6234 patients analyzed, 3770 (60.5%) of whom received ET and 2464 (39.5%) of whom received chemotherapy, multivariate analysis suggested that patients receiving ET plus anti-HER2 experienced improved OS compared with those receiving chemotherapy plus anti-HER2 (hazard ratio, 0.74; $p=0.004$).[47] Taken together, these studies suggest the potential utility of combined receptor blockade targeting HER2 and ER as a chemotherapy-free option in selected patients with HR+/HER2+ tumors. These trials also signal a need for further randomized studies testing this treatment paradigm, particularly in light of the fact that the SYSUCC-002 trial did not include the use of pertuzumab, and the National Cancer Database real-world analysis could have been biased by nonrandomized patient treatment assignment (e.g. in this study, patient assignment to ET plus anti-HER2 therapy was associated with older age, grade 1/2 disease, no visceral involvement, higher comorbidity scores, and being White).[46,47]

Table 1. Clinical trials of ET plus single or dual HER2-targeting approaches in postmenopausal women with HR+/HER2+ mBC.

Combination (Trial)	Phase	Cohort size	Key findings (Primary endpoint)
Trastuzumab + anastrozole *versus* anastrozole alone (TAnDEM)[42]	3	207	Median PFS • Trastuzumab + anastrozole: 4.8 months • Anastrozole alone: 2.4 months • Hazard ratio (95% CI): 0.63 (0.47–0.84) • $p = 0.0016$
Trastuzumab + letrozole *versus* letrozole alone (eLEcTRA)[41]	3	57	Median TTP • Trastuzumab + letrozole: 14.1 months • Letrozole alone: 3.3 months • Hazard ratio (95% CI): 0.67 (0.35–1.29) • $p = 0.23$
Lapatinib + letrozole *versus* placebo + letrozole[43]	3	219	Median PFS • Lapatinib + letrozole: 8.2 months • Placebo + letrozole: 3.0 months • Hazard ratio (95% CI): 0.71 (0.53–0.96) • $p = 0.019$
Lapatinib + trastuzumab + AI *versus* trastuzumab + AI *versus* lapatinib + AI (ALTERNATIVE)[44]	3	355	Median PFS • Lapatinib + trastuzumab + AI: 11 months • Trastuzumab + AI: 5.6 months • Hazard ratio[a] (95% CI): 0.62 (0.45–0.88) • $p = 0.0063$ • Lapatinib + AI: 8.3 months • Hazard ratio[b] (95% CI): 0.85 (0.62–1.17) • $p = 0.3159$
Trastuzumab + pertuzumab + AI *versus* trastuzumab + AI (PERTAIN)[45]	2	258	Median PFS • Trastuzumab + pertuzumab + AI: 18.9 months • Trastuzumab + AI: 15.8 months • Hazard ratio (95% CI): 0.65 (0.48–0.89) • $p = 0.007$

AI = aromatase inhibitor; ET = endocrine therapy; HER2 = human epidermal growth factor receptor 2; HR = hormone receptor; mBC = metastatic breast cancer; PFS = progression-free survival; TTP = time to progression.
[a]Lapatinib + trastuzumab + AI *versus* trastuzumab + AI.
[b]Lapatinib + trastuzumab + AI *versus* lapatinib + AI.

Role of cyclin D1 and CDK4/6 inhibitors in resistance of HR+/HER2+ mBC

Under normal conditions, mitogenic growth factors trigger cells to exit quiescence (G0) and enter the pre-DNA synthesis (G1) phase of the cell cycle before passing through the DNA synthesis (S), predivision (G2), and cell division (M) phases. Regulatory checkpoints throughout the cell cycle prevent unnecessary or erroneous cell division. In the early G1 phase, the cyclin D1–CDK4 kinase holoenzyme phosphorylates the retinoblastoma protein and other related proteins,[48] ultimately leading to the activation of E2F family transcription factors that facilitate S phase entry. Preclinical data have demonstrated that both the initiation and maintenance of the growth of HER2+ BCs require the presence of cyclin D1 and its activation of CDK4.[48–50] Activation of the oncogenes *Ras* and *Neu* (the rodent homologue of human HER2) upregulates cyclin D1 mRNA expression, as do ligand-activated ERs (cyclin D1 is a direct transcriptional target of ER), which indicates a key role for cyclin D1-CDK4/6 complexes in promoting breast tumorigenesis.[49,51,52] Moreover, amplification of the gene encoding cyclin D1 has also been identified in ~15%–20% of human BCs,[53] promoting uncontrolled cell proliferation.[51,54]

In vitro treatment of a panel of 44 human BC cell lines with palbociclib, a highly selective CDK4/6 inhibitor, found that ER+ and HER2+ cell lines were the most sensitive; inhibition resulted in prevention of proliferation and successive cell cycle arrest, supporting dependence of these tumors on CDK signaling.[53] Tamoxifen-resistant, ER+ xenografts and cancer cell lines (Table 2).

Table 2. Approved CDK4/6 inhibitors and their US indications in HR+/HER2– mBC.

Drug name	FDA-approved indication
Palbociclib (IBRANCE)[55]	HR+/HER2– advanced or mBC in combination with: • An AI as initial ET in postmenopausal women or in men • Fulvestrant in patients with disease progression following ET
Ribociclib (KISQALI)[56]	HR+/HER2– advanced or mBC in combination with: • An AI as initial ET • Fulvestrant as initial ET or following disease progression on ET in postmenopausal women or in men
Abemaciclib (VERZENIO)[57]	HR+/HER2– early BC with: • ET (tamoxifen or an AI) for the adjuvant treatment of adult patients with node-positive early BC at high risk of recurrence HR+/HER2– advanced or mBC: • In combination with an AI as initial ET in adult patients • In combination with fulvestrant in patients with disease progression following ET • As monotherapy in patients with disease progression following ET and chemotherapy in the metastatic setting

AI, aromatase inhibitor; ASCO, American Society of Clinical Oncology; BC, breast cancer; CDK4/6, cyclin-dependent kinase 4 and 6; ET, endocrine therapy; FDA, US Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; mBC, metastatic breast cancer; US, United States.

Cyclin D1 overexpression has been shown to mediate resistance to HER2-targeted therapies,[30] potentially through cyclin D1-facilitated ER transcriptional activity,[51] which reinforces the dependence of HR+ BC on cyclin D1. The observation that tumor cells surviving HER2 blockade retain high expression of cyclin D1 implies that inhibition of CDK4/6 could re-sensitize them to HER2-targeted therapy. Persistent CDK4/6 activity despite anti-HER2 therapy could sustain ongoing catalysis of mammalian target of rapamycin complex 1 (mTORC1), a cyclin-CDK downstream protein complex involved in the translation of proteins that allow cells to grow and proliferate, providing another means for ongoing S phase progression, and hence drug resistance. Combined CDK4/6 inhibition with HER2 blockade demonstrated synergistic activity in preclinical models of HER2+ BC resistant to HER2-targeted therapies.[30] This effect appears to be mediated through increased suppression of mTORC1.[30] As inhibition of CDK activity represents a highly selective anticancer strategy,[50] the addition of CDK4/6 inhibitors to standard anti-HER2 therapeutic regimens could represent a beneficial treatment strategy.

Ongoing clinical trials with CDK4/6 inhibitors in HR+/HER2+ BC

In an open-label phase 1/1b trial, the combination of palbociclib and T-DM1 was assessed as second- or later-line therapy in patients with HER2+ mBC, 66% of which were HR+/HER2+, and it was determined to be safe, tolerable, and active (overall response rate [ORR], 33%; median PFS, 6 months; median OS, 44.5 months).[58] The phase 2 PATRICIA trial (NCT02448420) assessed the combination of palbociclib and trastuzumab in patients with HER2+ BC pretreated with 2–4 previous lines of anti-HER2 therapy randomized to receive treatment with or without letrozole. The 6 month PFS rates in the subset of patients with HR+/HER2+ mBC treated concomitantly with and without letrozole were 46.4% and 42.9%, respectively, suggesting that the combination of CDK4/6 inhibition and anti-HER2 therapy exhibits promising activity in pretreated patients with advanced HR+/HER2+ disease treated both with and without ET.[59] In addition, luminal disease defined by prediction analysis of microarray 50 (PAM50) was independently associated with longer median PFS compared with non-luminal disease (10.6 months *versus* 4.2 months, respectively; adjusted hazard ratio 0.40; $p = 0.003$), reinforcing that within the HER2+ population, patients with a luminal subtype may benefit most from this therapeutic strategy.[59] The randomized, open-label, phase 2 monarcHER trial (NCT02675231) investigated the combination of abemaciclib, fulvestrant, and trastuzumab in patients with

advanced HR+/HER2+ disease who had received at least two prior HER2-targeted therapies. The combination achieved a median PFS of 8.3 months *versus* 5.7 months with standard-of-care chemotherapy plus trastuzumab (hazard ratio, 0.67; $p = 0.051$).[60] Recently, final results from the monarcHER trial presented at the ESMO Congress 2022 demonstrated numerically improved OS with abemaciclib plus trastuzumab with or without fulvestrant (31.1 months and 29.2 months, respectively) compared with chemotherapy plus trastuzumab (20.7 months).[61] Additionally, similar to results from PATRICIA, this study showed that luminal subtype tumors were associated with longer PFS [8.6 months *versus* 5.4 months (hazard ratio 0.54, 95% CI: 0.38, 0.79)] and OS [31.7 months *versus* 19.7 months (hazard ratio 0.68, 95% CI: 0.46, 1.00)] compared with non-luminal tumors. These findings further suggest that a chemotherapy-free regimen, such as with a CDK4/6 inhibitor, anti-HER2 targeted therapy, and fulvestrant, could be a viable treatment option for patients with HR+/HER2+ tumors.

Early-phase clinical trials of CDK4/6 inhibitors in HR+/HER2+ mBC have shown these regimens to have antitumor activity and manageable safety profiles. A phase 1b/2 study evaluating tucatinib (an anti-HER2 TKI), palbociclib, and letrozole in women who previously received at least 2 HER2-targeted treatments (NCT03054363) showed encouraging antitumor activity.[62] Among 26 patients with measurable disease, 8 (31%) had a partial response and 16 (62%) had stable disease; the combination also had a manageable safety profile. Interim results from the phase 2 DAP-Her-01 trial (NCT04293276) of the investigational CDK4/6 inhibitor dalpiciclib combined with pyrotinib – a pan-HER TKI currently available only in China – showed promising antitumor activity in patients with HR+ disease who had not received >1 line of systemic therapy in an advanced setting and who had not received prior treatment with CDK4/6 or HER2 inhibitors.[63] In that trial, 10 of 18 (56%) women achieved an objective response. Adverse events (AEs) were manageable, with diarrhea, leukopenia, neutropenia, anemia, and nausea most commonly reported. This combination could offer a completely oral, chemotherapy-free regimen for patients with HR+/HER2+ metastatic disease.

A few ongoing clinical trials are investigating the combination of CDK4/6 inhibition, ET, and anti-HER2 therapies in HR+/HER2+ mBC. The randomized, open-label, phase 3 PATINA trial (NCT02947685) is evaluating the role of CDK4/6 inhibition in HR+/HER2+ mBC following induction chemotherapy plus anti-HER2 therapy.[64] Eligible patients are randomized to receive standard anti-HER2 therapy (trastuzumab with or without pertuzumab) in combination with ET (AI or fulvestrant) with or without palbociclib as a maintenance strategy following completion of chemotherapy until disease progression. The primary endpoint is investigator-assessed PFS, with secondary endpoints of ORR, duration of response, clinical benefit, OS, safety, and quality of life; a comparison of PFS estimates according to *PIK3CA* mutation status is also included.[64,65] According to the most recently posted update, this trial has completed enrollment ($N = 496$), and results are awaited.

Finally, the randomized phase 3 Detect V/CHEVENDO trial (NCT02344472) is comparing chemotherapy *versus* ET in combination with dual HER2-targeted therapy (trastuzumab and pertuzumab) and ribociclib in patients with HR+/HER2+ mBC who have received no more than two prior chemotherapies and/or anti-HER2 therapies for metastatic disease.[66] In the chemotherapy arms, eligible patients receive trastuzumab and pertuzumab with chemotherapy (docetaxel, paclitaxel, or vinorelbine) initially, followed by maintenance ET and ribociclib. In the ET arms, eligible patients receive trastuzumab, pertuzumab, and ribociclib with ET (exemestane, fulvestrant, anastrozole, letrozole). The primary outcome is number of patients with AEs; other outcomes include quality-adjusted survival, PFS, ORR, AEs, OS, and occurrence of central nervous system metastases. As of this writing, the Detect V/CHEVENDO trial is recruiting patients.[67]

Future directions

Our understanding of the interactions among HER2-targeted therapies, ET, and CDK4/6 inhibitors continues to evolve. Data suggest that immune evasion may contribute to the growth of HR+/HER2+ tumors and that the tumor microenvironment may influence sensitivity to systemic treatments. Thus, immune enrichment may promote an improved response to anti-HER2 therapies.[68] As an example, a retrospective analysis of the CLEOPATRA trial found that a higher level (>20%) of tumor-infiltrating lymphocytes (TILs)

in pretreatment tumor samples was associated with improved OS.[69] Further, preclinical data suggest that CDK4/6 inhibitors may promote T-cell activation and enhance T-cell activity.[70,71] Studies have also suggested that TILs could have important roles in disease progression *via* ER signaling activation (reviewed by Segovia-Mendoza and Morales-Montor[72]), as well as estrogen-induced effects in other immune cell subpopulations.[73] This further highlights the critical importance of identifying complementary treatment strategies to improve outcomes. Changes in proliferation, apoptosis, or both occur with combined CDK4/6 and HER2 inhibition,[30] lending support for therapeutic combinations including CDK4/6 inhibitors to influence cancer cell immunogenicity, apoptosis, and differentiation. As with all treatment combinations, safety must be carefully considered in addition to efficacy. Common safety signals for all discussed approved treatments can be found in Table 3.

Table 3. Safety signals for discussed treatments in advanced BC.

Drug category	Drug name	Common safety signals	
		Warnings and precautions	**Most common AEs**
CDK4/6 inhibitors			
Class: Kinase inhibitors	Palbociclib (IBRANCE)[55]	• Neutropenia • ILD/pneumonitis	Most common AEs (⩾10%) were neutropenia, infections, leukopenia, fatigue, nausea, stomatitis, anemia, alopecia, diarrhea, thrombocytopenia, rash, vomiting, decreased appetite, asthenia, and pyrexia
	Abemaciclib (VERZENIO)[57]	• Diarrhea • Neutropenia • ILD/pneumonitis • Hepatotoxicity • Venous thromboembolism	Most common AEs (⩾20%) were diarrhea, neutropenia, nausea, abdominal pain, infections, fatigue, anemia, leukopenia, decreased appetite, vomiting, headache, alopecia, and thrombocytopenia
	Ribociclib (KISQUALI)[56]	• ILD/pneumonitis • Severe cutaneous adverse reactions • QT interval prolongation • Hepatotoxicity • Neutropenia	Most common AEs (⩾20%) were neutropenia, nausea, infection, fatigue, diarrhea, leukopenia, vomiting, alopecia, headache, constipation, rash, and cough
EGFR inhibitor			
Class: TKI	Gefitinib (IRESSA)[74]	• ILD • Hepatotoxicity • Gastrointestinal perforation • Diarrhea • Ocular disorders including keratitis • Bullous and exfoliative skin disorders	Most common AEs (⩾20%) were skin reactions and diarrhea
Endocrine therapy			
Class: AI	Letrozole (FEMARA)[75]	• Osteopenia • Cholesterol • Fatigue	Most common AEs (⩾20%) were hot flashes, arthralgia, flushing, asthenia, edema, headache, dizziness, hypercholesterolemia, sweating increase, bone pain, and musculoskeletal pain
	Exemestane (AROMASIN)[76]	• Osteopenia	Most common AEs for patients with advanced BC were hot flashes, nausea, fatigue, increased sweating, and increased appetite

(Continued)

Table 3. (Continued)

Drug category	Drug name	Common safety signals	
		Warnings and precautions	**Most common AEs**
	Anastrozole (ARIMIDEX)[77]	• Cardiovascular events • Osteopenia • Cholesterol	Most common AEs (⩾20%) in patients with advanced BC were hot flashes, nausea, asthenia, pain, headache, back pain, bone pain, increased cough, dyspnea, pharyngitis, and peripheral edema
Class: SERM	Tamoxifen (NOLVADEX)[78]	• Hypercalcemia • Uterine malignancies • Thromboembolic events • Hepatotoxicity • Effects on the eye	Most common AE in patients with mBC was hot flashes; other infrequent AEs were hypercalcemia, peripheral edema, distaste for food, pruritus vulvae, depression, dizziness, light-headedness, headache, hair thinning and/or partial hair loss, and vaginal dryness
HER2 inhibitors			
Class: mAb	Trastuzumab (HERCEPTIN)[79]	• Cardiomyopathy • Infusion reactions • Neutropenia • Pulmonary toxicity	Most common AEs (⩾10%) for patients with mBC were fever, chills, headache, infection, congestive heart failure, insomnia, cough, and rash
	Pertuzumab (PERJETA)[80]	• Left ventricular dysfunction • IARs/hypersensitivity reactions/anaphylaxis	Most common AEs (>30%) in combination with trastuzumab and docetaxel were diarrhea, alopecia, neutropenia, nausea, fatigue, rash, and peripheral neuropathy
Class: ADC	T-DM1, ado-trastuzumab emtansine (KADCYLA)[22]	• Pulmonary toxicity • IRRs/hypersensitivity reactions • Thrombocytopenia • Neurotoxicity	Most common AEs (⩾25%) for mBC were fatigue, nausea, musculoskeletal pain, thrombocytopenia, hemorrhage, headache, increased transaminases, constipation, and epistaxis
	Fam-trastuzumab deruxtecan-nxkl (ENHERTU)[24]	• ILD/pneumonitis • Neutropenia • Left ventricular dysfunction	Most common AEs (⩾20%) in patients with BC were nausea, decreased blood cell count, decreased hemoglobin, decreased neutrophil count, increased AST, fatigue, decreased lymphocyte count, vomiting, decreased platelet count, increased ALT, increased blood alkaline phosphatase, alopecia, constipation, hypokalemia, decreased appetite, diarrhea, musculoskeletal pain, increased transaminases, respiratory infection, headache, and abdominal pain
Class: TKI	Lapatinib (TYKERB)[81]	• Left ventricular dysfunction • Hepatotoxicity • Diarrhea • ILD/pneumonitis • QT interval prolongation	Most common AEs (⩾20%) in combination with letrozole were diarrhea, rash, nausea, and fatigue
	Neratinib (NERLYNX)[81]	• Diarrhea • Hepatotoxicity	Most common AEs (⩾5%) were diarrhea, nausea, abdominal pain, fatigue, vomiting, rash, stomatitis, decreased appetite, muscle spasms, dyspepsia, AST or ALT increase, nail disorder, dry skin, abdominal distention, epistaxis, weight decrease, and urinary tract infection
	Tucatinib (TUKYSA)[82]	• Diarrhea • Hepatotoxicity	Most common AEs (⩾20%) were diarrhea, palmar-plantar erythrodysesthesia, nausea, fatigue, hepatotoxicity, vomiting, stomatitis, decreased appetite, abdominal pain, headache, anemia, and rash

(Continued)

Table 3. (Continued)

Drug category	Drug name	Common safety signals	
		Warnings and precautions	**Most common AEs**
PI3K/AKT pathway inhibitors			
Class: TKI	Alpelisib (PIQRAY)[82]	• Hypersensitivity reactions • Severe cutaneous reactions • Hyperglycemia • ILD/pneumonitis • Diarrhea	Most common AEs (⩾20%) were glucose increase, creatinine increase, diarrhea, rash, lymphocyte count decrease, GGT increase, nausea, ALT increase, fatigue, hemoglobin decrease, lipase increase, decreased appetite, stomatitis, vomiting, weight decrease, calcium decrease, glucose decrease, aPTT prolongation, and alopecia

ADC, antibody-drug conjugate; AE, adverse event; AI, aromatase inhibitor; AKT, protein kinase B; ALT, alanine transaminase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BC, breast cancer; CDK4/6, cyclin-dependent kinase 4 and 6; EGFR, epidermal growth factor receptor; GGT, gamma-glutamyl transferase; HER2, human epidermal growth factor receptor 2; IAR, infusion-associated reaction; ILD, interstitial lung disease; IRR, infusion-related reaction; mAb, monoclonal antibody; mBC, metastatic breast cancer; PI3K, phosphatidylinositol-3-kinase; SERM, selective estrogen receptor modulator; TKI, tyrosine kinase inhibitor.

As outlined above, ER activation, whether through mutations in ER or its downstream effectors, may lead to dysregulation of downstream PI3K/AKT/mTOR pathways and subsequent resistance to current treatment strategies. Mutations in the gene coding for ER (*ESR1*) have been found in up to 42% of HR+ advanced BCs, with the majority of these mutations being in a hotspot region within the ligand binding domain that functional studies have shown leads to constitutive, ligand-independent ER activation.[83] These mutations are enriched following AI treatment in the metastatic setting and are associated with shorter PFS, indicating they may lead to ET resistance and alterations in downstream signaling.[83,84]

The PI3K enzyme and its principal downstream target molecule, AKT, regulate the cell cycle, growth, proliferation, and modulate energy metabolism.[85] Hyperactivation of this pathway contributes to primary or acquired resistance to anti-HER2 therapy and represents an alternative survival pathway for cancer cells.[86,87] In a study of HER2+ BC tissue samples, 71% of trastuzumab-refractory tumors had activating mutations in the PI3K catalytic subunit (*PIK3CA*) and/or absent or reduced expression of tumor suppressor *PTEN* compared with 44% of a cohort of HER2+ BC not exposed to trastuzumab.[88] Mutations have also been identified in *PIK3CA* in ~17% of HER2+ and ~21% of HR+ BCs,[89] loss of function of *PTEN* also occurs in 13%–35% of BCs.[87] In the CLEOPATRA trial, patients harboring a *PIK3CA* mutation had a shorter PFS than those with wild-type *PIK3CA*.[90] Inhibitors of the PI3K/AKT/mTOR pathway could therefore be utilized as new agents to help avoid resistance to current therapies. The phase 3 SOLAR-1 trial (NCT02437318) compared the combination of the PI3K/AKT pathway antagonist alpelisib plus fulvestrant with placebo plus fulvestrant in patients with HR+/HER2− mBC with and without *PIK3CA* mutations. PFS with alpelisib plus fulvestrant was significantly improved compared with placebo plus fulvestrant (11.0 months *versus* 5.7 months, respectively; $p < 0.001$) in patients with PIK3CA mutations.[91] The most common grade 3–4 AEs with alpelisib plus fulvestrant treatment were hyperglycemia and rash, with grade 3 diarrhea reported in 6.7% of patients compared with 0.3% in the placebo plus fulvestrant group. Other trials are now translating these findings to the HER2+ mBC setting; for example, the ongoing open-label, phase 1b IPATHER trial (NCT04253561), is assessing the safety and preliminary efficacy of ipatasertib, a PI3K/AKT pathway antagonist, in combination with trastuzumab and pertuzumab with or without ET in patients with HER2+ mBC with a *PIK3CA* mutation.[92] Results for six patients as of the data cutoff of February 2021 indicated that the combination was tolerable; in addition, one patient achieved a partial response and the other five had stable disease. Furthermore, the ongoing double-blind, randomized, placebo-controlled EPIK-B2 phase 3 trial (NCT04208178) is evaluating the efficacy and safety of alpelisib with trastuzumab and pertuzumab maintenance therapy for HER2+ advanced BC.[93]

Other ongoing studies are investigating novel combinations of HER2-targeted therapy with palbociclib and a selective ER degrader. Preliminary results from a study of neoadjuvant therapy with trastuzumab, pertuzumab, and palbociclib in combination with fulvestrant in women with HR+/HER2+ BC (the NA-PHER2 study; NCT02530424) indicate that this combination has a significant impact on Ki-67 expression 2 weeks after treatment.[94] The investigational anti-HER2 monoclonal antibody zanidatamab is being studied in locally advanced/mBC in combination with fulvestrant and palbociclib in a phase 2 trial that is currently recruiting patients (NCT04224272).

Conclusions

The HR+/HER2+ BC subtype represents a distinct clinical entity from HR−/HER2+ BC, and as such, warrants individualized options to maximize clinical outcomes. Given the heterogeneity among HR+/HER2+ tumors and the pathway crosstalk-mediated acquisition of treatment resistance, combined receptor blockade targeting both HR and HER2 signaling pathways merits further investigation. Promising preclinical and early-phase clinical trial data suggest that combination treatment with anti-HER2 therapies and ET, with or without CDK4/6 inhibition, may provide superior efficacy compared with targeted HER2 blockade alone. Results from ongoing trials (e.g. PATRICIA, IPATHER) designed to test this hypothesis and to compare HR/HER2 blockade with CDK4/6 and HR/HER2 blockade are eagerly awaited to inform the future treatment of HR+/HER2+ mBC.

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Not applicable.

Consent for publication

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Author contributions

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