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Academia–Pharma Intersect: Breast Cancer

Targeting Signal Transduction Pathways in Metastatic Breast Cancer: A Comprehensive Review

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ABSTRACT

Greater understanding of the underlying etiology and biology of breast cancer is enabling the clinical development of targeted therapies for metastatic breast cancer (MBC). Following the successful introduction of trastuzumab, the first human epidermal growth factor receptor (HER) biologically targeted therapy to become widely used in MBC patients, other agents have been developed. Novel agents include monoclonal antibodies such as pertuzumab, which bind to receptors on the cell surface, and tyrosine kinase inhibitors (TKIs) such as lapatinib, which target intracellular pathways such as that of the epidermal growth factor receptor. There is also growing clinical experience with antiangiogenic agents, particularly in combination with chemotherapy. These include the monoclonal antibody bevacizumab, which targets vascular endothelial growth factor receptor, and multitargeted TKIs with antiangiogenic and antiproliferative activities, such as sunitinib. Combination treatment with multiple agents targeting both the HER family and angiogenic pathways (e.g., trastuzumab plus bevacizumab) is also showing activity in the clinical setting. Despite recent advances, there are unanswered questions regarding the management of MBC with targeted agents. Future studies are necessary to determine the optimal combinations, doses, and schedules required to maximize clinical activity while minimizing toxicity. Despite the temptation to use a targeted agent in all patients, identification of patient subgroups most likely to benefit must be a key goal and will be critical to the successful future use of these treatments. The aim of this review is to summarize some of the key signaling pathways involved in tumor progression and some of the novel therapies that are in development for MBC.
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INTRODUCTION

The mechanisms underlying the development of breast cancer are complex and vary among individual tumors [1]. These include genetic and epigenetic alterations, and resulting changes in the activity of signaling pathways.

Mutations or epigenetic functional inactivation of tumor suppressor genes may contribute to the early development of some tumors, and alterations in proto-oncogenes may also be involved [2]. Altered patterns of gene expression are associated with corresponding variations in growth rates

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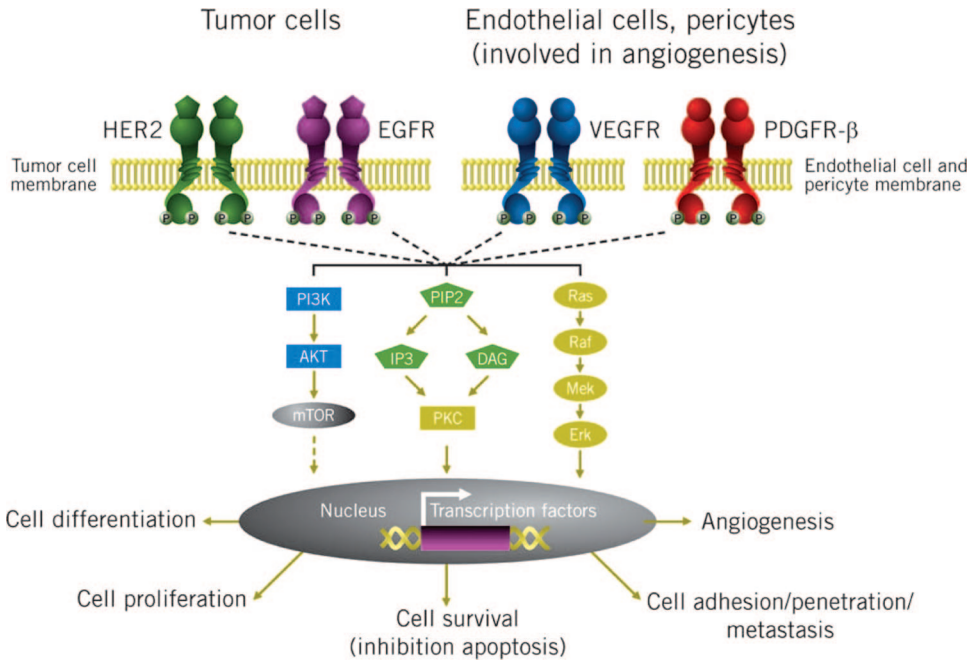

Figure 1. Key targets for breast cancer treatment.

Abbreviations: DAG, diacyl glycerol; EGFR, epidermal growth factor receptor; ERK, extracellular signal–related kinase kinase; HER-2, human epidermal growth factor receptor 2; IP3, inositol 1,4,5-trisphosphate; mTOR, mammalian target of rapamycin; PDGFR-β, platelet-derived growth factor receptor β; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; VEGFR, vascular endothelial growth factor receptor.

and cellular composition [3]. Analyzing these patterns of gene expression can help to define tumor subtypes. Two of the subtypes so far identified are those with gene expression characteristics typical of basal epithelial cells (which are predominantly estrogen receptor [ER]$^-$) and those with gene expression characteristics typical of luminal epithelial cells (which are predominantly ER$^+$) [3, 4]. Different patterns of gene expression are also associated with differing prognoses, and genetic assay techniques are increasingly being used to provide information on outcome, including the risk for tumor recurrence and whether an individual is likely to benefit from a particular chemotherapy [5]. Typically, tumors of the ER$^-$, basal subtype are associated with shorter relapse-free and overall survival times than those of the ER$^+$, luminal subtype [4]. Altered patterns of gene expression can also influence the activity of specific signaling pathways. Variations in the pathways associated with the human epidermal growth factor receptor (HER) family, which are encoded by genes on different chromosomes and regulate normal breast growth and development, appear to be particularly important, not only in tumor development but also in treatment efficacy [6, 7]. Given the complex and varied factors that influence the development of breast cancer, and the use of increasingly sophisticated genetic analysis techniques, it is likely that more refined tumor subtypes and their associated prognoses will be identified [1, 8].

Current treatment strategies for metastatic breast cancer (MBC) depend upon patient and tumor classification; menopausal status, hormone receptor status, and HER-2 status may all be considered, as may site of metastatic disease (bone or soft tissue). The choice of treatment in metastatic disease is still a matter of debate, but usually involves systemic endocrine therapy or cytotoxic chemotherapy, with an anti–HER-2 agent when appropriate. Novel agents are needed because many of the current therapies have limitations. These include drug resistance, lack of target receptor expression in tumors (e.g., only 25% of breast cancer tumors have HER-2 expression), and relatively small improvements in survival [9–12]. In addition, as more treatment options become available in the first-line and adjuvant settings, there is less clarity about choices for patients with metastatic or refractory disease. Advances in the understanding of the etiology and biology of breast cancer have identified key targets among the multiple signaling pathways involved in the development, growth, and survival of breast cancer cells (Fig. 1). As such, targeted therapies are among the most promising new agents for the treatment of breast cancer.

This review focuses on some of the mechanisms and pathways influencing tumor cell proliferation, survival, and invasiveness that are being exploited to develop novel therapies for the treatment of MBC. Targets currently identified

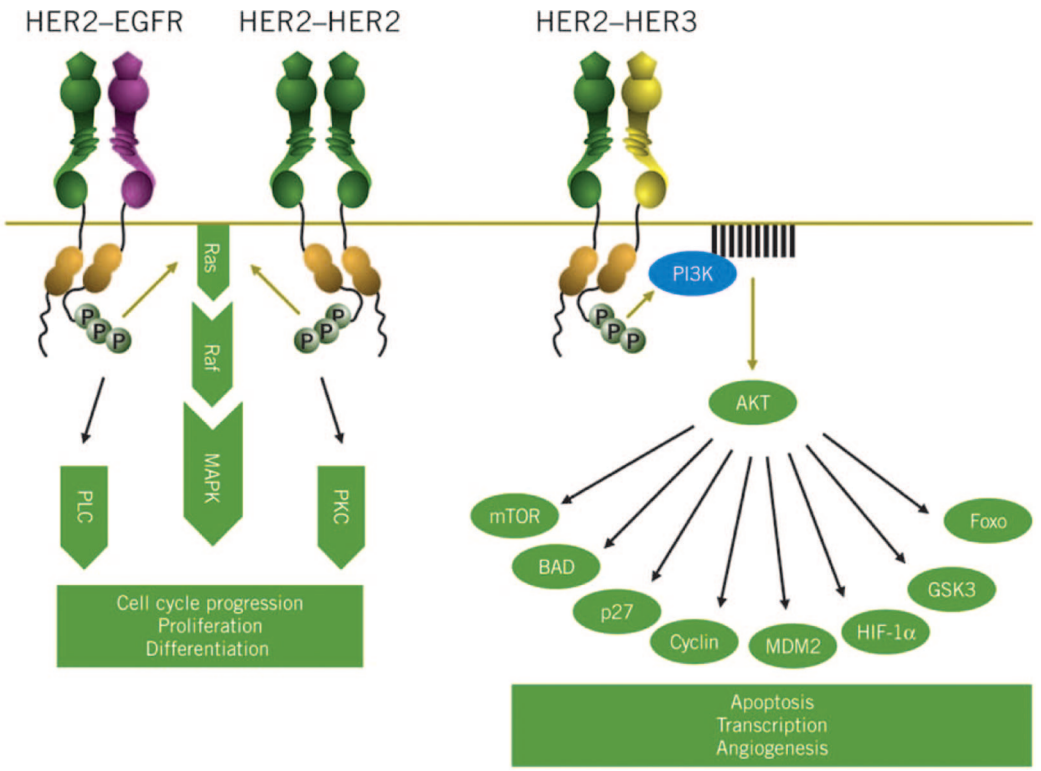

Figure 2. HER-activated signaling pathways.

Abbreviations: BAD, Bcl-2-associated death promoter; EGFR, epidermal growth factor receptor; GSK3, glycogen synthase kinase 3; HER-2, human epidermal growth factor receptor 2; HIF-1α, hypoxia inducible factor 1α; MAPK, mitogen-activated protein kinase; MDM2, murine double minute 2; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C. Adapted from Atalay G, Cardoso F, Awada A et al. Novel therapeutic strategies targeting the epidermal growth factor receptor (EGFR) family and its downstream effectors in breast cancer. Ann Oncol 2003; 14:1346–1363, by permission of Oxford University Press.

include HER family members and members of the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and apoptotic signaling pathways. Factors modulating angiogenesis are additional targets for therapy, and recent clinical developments with antiangiogenic therapies are also reviewed. There appears to be extensive crosstalk between the pathways driving tumorigenic processes, and this provides a good rationale for inhibiting multiple pathways and processes with multitargeted agents, either as single agents or in combination.

The HER Family

The HER family consists of four closely related tyrosine kinase receptors: HER-1 (also termed epidermal growth factor receptor [EGFR] or ErbB-1), HER-2 (also termed ErbB-2 or HER-2/neu), HER-3 (ErbB-3), and HER-4 (ErbB-4) [13]. Through their interconnected cellular signaling network, the HER family regulates diverse biological processes, including cell proliferation, differentiation, and survival [13, 14], and plays a key role in the development and progression of breast cancer [13, 15, 16]. Expression of HER family members in breast cancer tumors has a significant impact on tumor aggressiveness and patient survival. HER-1 and HER-2 are expressed in approximately 16%–48% and 25%–30% of breast cancer tumors, respectively, and their expression correlates with a more aggressive disease course, shorter survival time, and higher risk for resistance to endocrine therapies [9, 10, 17–23]. HER-3 expression, observed in approximately 18% of tumors, also correlates with shorter overall survival [19]. Interestingly, expression of HER-4 (found in approximately 12% of tumors) has been associated with more favorable tumor characteristics and longer survival [18, 19].

Each HER receptor has an extracellular domain involved in ligand binding, a helical transmembrane segment, and an intracellular protein tyrosine kinase domain [13, 24]

(Fig. 2). On ligand binding, the extracellular domains of the receptors undergo conformational changes, which allows them to form homodimers (consisting of two identical receptors) or heterodimers (consisting of two different receptors) of the HER family [13, 24] (Fig. 2). More than 10 ligands have so far been identified that bind to HER-1, HER-3, and HER-4, including epidermal growth factor (EGF), transforming growth factor α, amphiregulin, betacellulin, epiregulin, heparin-binding EGF, and neuregulin 1 through neuregulin 4 [13]. Putative ligands of HER-2 have been characterized, but no specific ligand has yet been identified [24]. This has clinical implications in terms of there being no alternative approach to blocking this pathway, and this may be related to the development of resistance to HER-2 blockade.

The specific receptors involved in each dimer affect the type and number of downstream effectors activated, and also influence the downregulation mechanism for the ligand-bound receptors [24]. Dimerization of HER receptors induces phosphorylation of their intracellular tyrosine kinase domains, which provide docking sites for adaptor proteins and signaling enzymes [24]. These molecules act as a link between membrane receptor kinases and "downstream" intracellular protein kinases, which results in the activation of multiple signaling pathways, of which the MAPK and PI3K pathways are probably the best understood [24] (Fig. 2). Given the absence of known ligands for HER-2, and lack of tyrosine kinase activity of HER-3, it is assumed that these receptors must form heterodimers with another member of the HER family in order to activate signaling [24]. HER-2 is the preferred dimerization and signaling partner for all other members of the HER family, and it appears to function mainly as a coreceptor, increasing the affinity of ligand binding to dimerized receptor complexes [24, 25]. With their multiple ligands, many dimerization combinations, and large number of downstream effectors, the HER family mediates an extensive range of signals, controlling a variety of cellular processes, including cellular proliferation, apoptosis, and angiogenesis [24, 26] (Fig. 2).

Targeted Therapies Directed at the HER Family of Receptors

Numerous agents targeting individual members of the HER family have been developed for use in the treatment of breast cancer. Table 1 summarizes those that are licensed or in phase III clinical development. Existing therapeutic approaches have largely focused on two classes of agents. The first comprises monoclonal antibodies that bind to extracellular regions of HER to interfere with receptor function (e.g., trastuzumab, pertuzumab, and a number of pan-HER inhibitors). Trastuzumab binds to the juxtamembrane region of HER-2 with high specificity, but it is not currently known how it specifically interferes with HER-2 function [27]. Pertuzumab is the first in a class of HER-2 dimerization inhibitors. Binding to HER-2 inhibits its dimerization with other HER receptors and this is thought to result in slowed tumor growth [28]. The second class of HER-targeted agents comprises the small molecule tyrosine kinase inhibitors (TKIs) that inhibit enzyme function of HER family members intracellularly. Oral TKIs include lapatinib, neratinib (both inhibit HER-1 and HER-2), erlotinib and gefitinib that target the intracellular domain of HER-1, and the irreversible pan-HER inhibitors PF-00299804 and canertinib, which inhibit the kinase signaling of multiple HER family members [29, 30].

Extracellular Targeted Therapies: Monoclonal Antibodies

Trastuzumab. Trastuzumab, administered as an i.v. infusion, is approved in the U.S. and Europe for the treatment of HER-2–overexpressing MBC [31]. It is standard-of-care treatment for MBC patients with HER-2–overexpressing tumors, both as first-line treatment in combination with chemotherapy and as a single agent in women who have HER-2–overexpressing MBC that has progressed after chemotherapy for metastatic disease [32, 33]. Trastuzumab is approved for the adjuvant treatment of HER-2^+, node-negative (ER^-/progesterone receptor $[PgR]^-$ or with one high-risk feature) or node-positive breast cancer, either in combination with chemotherapy or as a single agent following multimodality anthracycline-based treatment.

Studies have evaluated a range of different trastuzumab-based combination regimens for the first-line treatment of MBC (Table 2A) [34–48]. Additionally, a number of trials are ongoing investigating trastuzumab in combination with hormonal therapy in MBC patients [49, 50].

When administered as a single agent, trastuzumab has documented efficacy as a first-line therapy, with response rates typically in the range of 23%–33% [51–53]. However, no benefit has been observed treating patients with single-agent trastuzumab followed by chemotherapy with or without trastuzumab on progression [35, 41]. Of those patients with MBC who do achieve an initial response, many experience disease progression within 12 months as a result of the high proportion of HER-2–overexpressing tumors that have intrinsic resistance to this agent [54]. However, changing the traditional treatment paradigm in patients progressing on trastuzumab and administering further trastuzumab-based therapy beyond disease progression may have clinical benefit [55, 56]. This "treatment beyond progres-

Table 1. Therapies currently licensed or in phase III clinical development for MBC

Target pathway	Agent	Specific target	Drug class	Licensing status
HER	Trastuzumab (Herceptin®)	HER-2	mAb	MBC: In combination with paclitaxel for first-line treatment of HER-2$^+$ patients; as a single agent for treatment of HER-2$^+$ patients who have received one or more chemotherapy regimens for MBC
				Adjuvant: For HER-2–overexpressing node-positive or node-negative breast cancer as part of a regimen consisting of doxorubicin, cyclophosphamide, and either paclitaxel or docetaxel; with docetaxel and carboplatin; as a single agent following multimodality anthracycline-based therapy
	Pertuzumab (Omnitarg®)	HER-2	mAb	Not currently approved in breast cancer
	Lapatinib (Tykerb®/Tyverb®)	HER-1 and HER-2	TKI	MBC: In combination with capecitabine in HER-2$^+$ patients who have progressed following trastuzumab, an anthracycline, and a taxane (application submitted for first-line therapy in combination with hormonal therapy)
	Neratinib	HER-1 and HER-2	TKI	Not currently approved in breast cancer
Angiogenesis	Bevacizumab (Avastin®)	VEGF	mAb	MBC: In combination with docetaxel (EU) or paclitaxel (EU and U.S.) for first-line treatment
	Sunitinib malate (SUTENT®)	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, Kit, RET, FLT-3, CSF-1R	TKI	Not currently approved in breast cancer

Many other targeted agents are currently being investigated in early phase I/II clinical trials of MBC (e.g., pazopanib, axitinib, sorafenib, everolimus).
Abbreviations: CSF-1R, colony-stimulating factor 1 receptor; EU, European Union; FLT-3, FMS-like tyrosine kinase 3; HER, human epidermal growth factor receptor; mAb, monoclonal antibody; MBC, metastatic breast cancer; PDGFR, platelet-derived growth factor receptor; RET, glial cell-derived neurotrophic factor (REarranged during Transfection); TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

sion" approach is increasingly being studied in clinical trials by combining trastuzumab either with chemotherapy [43, 57, 58] or with another targeted agent, such as the TKI lapatinib [59, 60] and the HER-2 dimerization inhibitor pertuzumab [28, 61] (Table 2B).

A phase III evaluation of continuing trastuzumab and capecitabine versus capecitabine alone in patients (n = 156) with HER-2$^+$ MBC who had progressed during trastuzumab treatment found that the combination led to a longer time to progression (TTP), by nearly 3 months, than with capecitabine alone (8.2 months versus 5.6 months; p = .034) [43]. In addition, recent phase II data showed how 50% of patients who had progressed on trastuzumab therapy benefited from combination treatment with pertuzumab and trastuzumab; combination treatment resulted in an overall response rate (ORR) of 24.2% (complete response rate, 7.6%; partial response [PR] rate, 16.7%; rate of stable disease [SD] >6 months, 25.8%) and a progression-free survival (PFS) duration of 24 weeks [28, 45]. The combination appeared to be well tolerated, and no patients were withdrawn as a result of toxicities. A phase III clinical trial (CLEOPATRA) evaluating trastuzumab plus chemotherapy with and without pertuzumab for the first-line treatment of HER-2$^+$ MBC is currently ongoing [45]. Interestingly, an evaluation of trastuzumab use beyond disease progression by the National Comprehensive Cancer Network found that, of the total 165 patient cohort, 46 patients stopped first-line treatment because of disease progression. Of those 46 patients, 74% continued to receive trastuzumab as part of second-line therapy and nine of 46 (19.6%) patients were treated in a clinical trial [48].

Trastuzumab-DM1 (T-DM1) is an anti–HER-2 antibody drug conjugate comprising trastuzumab linked to the maytansine derivative DM1. Combining these two agents facilitates anti–HER-2 activity as well as targeted intracellular delivery of a potent cytotoxic agent. Single-agent T-

Table 2. Summary of key phase II and III trials of: **(A)** trastuzumab in combination with chemotherapy (first-line treatment) and **(B)** trastuzumab treatment beyond progression

Trial	Phase	Patient characteristics	Treatment regimen	Primary endpoint	ORR	PFS/TTP	OS	Safety
A. Trastuzumab + chemotherapy								
[34]	II	HER-2$^+$ (n = 186)	D alone versus D + T	ORR	ORR, 34% (D) versus 61% (D + T) (p = .0002)	Median TTP, 6.1 mos (D) versus 11.7 mos (D + T) (p = .0001)	Median OS, 22.7 mos (D) versus 31.2 mos (D + T) (p = .0325)	Combination treatment produced little additional toxicity
HERTAX [35]	II	HER-2$^+$ (n = 99)	Sequential T + D versus combination T + D	PFS	ORR, 50% with sequential treatment versus 73% with combination (p = .02)	Median PFS, 10.8 mos (sequential) versus 9.4 mos (combination); HR, 1.21 (p = .42)	Median OS, 20.2 mos versus 30.5 mos; HR, 1.45 (p = .15)	More grade 3 or 4 toxicity with combination treatment
[36]	II	HER-2$^+$ (n = 30)	T + nab-paclitaxel + C	ORR	ORR, 53%; CR, 2 (7%); PR, 14 (47%)	Median TTP, 15.9 mos (95% CI, 7.3–28.0); median DR, 28 mos	Not available	Well tolerated
[37]	II	HER-2$^+$ (n = 30)	Pegylated liposomal doxorubicin + T	ORR	CR/PR, 52%	Median PFS, 12 mos	Not available	Cardiotoxicity (asymptomatic decreases in LVEF)
[33]	III	HER-2$^+$ (n = 469)	Standard chemotherapy alone versus standard chemotherapy + T	TTP	ORR, 32% versus 50% (+T) ($p < .001$)	Median TTP, 4.6 mos versus 7.4 mos (+T) ($p < .001$)	Median OS, 20.3 mos versus 25.1 mos (+T) (p = .046)	Most important AE, cardiotoxicity
TRAVIOTA [38]	III	HER-2$^+$ (n = 81)	T + V versus T + PX/D	ORR	ORR, 51% (T + V) versus 40% (T + PX/D) (p = .37)	Median TTP, 8.5 mos (T + V) and 6.0 mos (T + PX/D) (p = .09)	Not available	Comparable safety profiles
[39]	III	HER-2$^+$ (n = 196)	C + T + PX versus T + PX	ORR	ORR, 52% (C + T + PX) versus 36% (T + PX) (p = .04)	Median PFS, 10.7 mos (C + T + PX) versus 7.1 mos (T + PX) (p = .03)	Not available	More grade 4 neutropenia with carboplatin regimen
[40]	III	HER-2$^+$ (n = 263)	C + T + D versus T + D	ORR	ORR, 73% in both arms	Median TTP, 10.4 mos (C + T + D) versus 11.1 mos (T + D) (p = .57)	Not available	Effective and well tolerated; no significant cardiotoxicity
[41]	III	HER-2$^+$ (n = 105)	Combination T + D versus sequential T → T + D	PFS and OS	ORR, 68% (combination T + D) versus 15% (sequential T alone) versus 47% (sequential T + D); significant difference for combination versus sequential T + D	PFS, 14.6 mos (combination T + D) versus 3.7 mos (sequential T alone) versus 12.4 mos (sequential T + D); significant difference for sequential T versus sequential T + D	OS, significant difference for combination versus sequential T + D	No difference in LVEF; comparable safety profile

(continued)

Table 2. (Continued)

Trial	Phase	Patient characteristics	Treatment regimen	Primary endpoint	ORR	PFS/TTP	OS	Safety
CHAT [42]	III	HER-2$^+$ (n = 222)	X + T + D versus T + D	ORR	ORR, 71% (X + T + D) versus 73% (T + D) (p = .717)	Median TTP, 18.2 mos (X + T + D) versus 13.8 mos (T + D) (p = .045)	OS, not mature at yr 1 and yr 2; survival higher with X + T + D	No greater cardiotoxicity with triple combination
B. Trastuzumab treatment beyond progression								
[43]	III	HER-2$^+$ (n = 156)	Continuing T + X versus X	ORR	ORR, 48% (T + X) versus 27% (X) (p = .0115); CBR, 75% (T + X) versus 54% (X) (p = .007)	Median TTP, 8.2 mos (T + X) versus 5.6 mos (X) (p = .0338)	Median OS, 25.5 mos (T + X) versus 20.4 mos (X) (p = .257)	Similar toxicity for T + X versus X alone
[44]	II	HER-2$^+$ (n = 31)	TP + T	ORR	ORR: PR, 7 patients (26%), SD ≥4 mos, 5 patients (19%); CBR, 17 patients (63%)	Not available	Not available	Well tolerated
[28, 45]	II	HER-2$^+$ (n = 66)	T + PZ	ORR	ORR, 24% (CR, 5 patients; PR, 11 patients); SD >6 mos, 17 patients; CBR, 50%; DR, 25 wks	Not available	Not available	Well tolerated
[46]	I	HER-2$^+$ (n = 27)	R + T + PX	ORR	CR, 5%; PR, 36%; SD, 50%; DCR (PR/SD >16 wks), 77%. In 11 patients with taxane and T-resistant tumors: CR, 11%; PR, 56%; SD, 33%; DCR, 100%	Not available	Not available	Well tolerated (very low incidence and severity of AEs)
[47]	I	HER-2$^+$ (n = 37)	R + T + V, 5 mg daily (n = 17) versus weekly 20 mg/day (n = 6) versus weekly 30 mg/day (n = 14)	ORR	Of 34 patients: 5 mg daily: CR, 1; PR, 2; SD, 9; weekly 20 mg/day: PR, 1; SD, 3; weekly 30 mg/day: PR, 2; SD, 9; CBR, 50%	Not available	Not available	Generally well tolerated
[48]	NA	HER-2$^+$	T (retrospective analysis)	Not available	74% (46 of 62 patients) continued to receive T as part of second-line treatment following PD	Not available	Not available	Not available

Abbreviations: AE, adverse event; C, carboplatin; CBR, clinical benefit rate; CI, confidence interval; CR, complete response; D, docetaxel; DCR, disease control rate; DR, duration of response; HER, human epidermal growth factor receptor; HR, hazard ratio; L, lapatinib; LVEF, left ventricular ejection fraction; NA, not applicable; NCCN, National Comprehensive Cancer Network; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; PX, paclitaxel; PZ, pertuzumab; R, RAD001; SD, stable disease; T, trastuzumab; TP, tanespimycin; TTP, time to progression; V, vinorelbine; X, capecitabine.

DM1 was well tolerated and active (ORR, 25%; clinical benefit rate [CBR], 34.8%) and no dose-limiting cardiotoxicity was observed in a phase II study of 112 patients with pretreated MBC [62].

Limitations of Trastuzumab Therapy. Trastuzumab is unable to penetrate the blood–brain barrier [63], and overexpression of HER-2 is known to be associated with a greater risk for central nervous system (CNS) metastases [64]. Patients with HER-2^+ MBC treated with trastuzumab appear to be at greater risk for developing CNS metastases than those who do not receive trastuzumab therapy [65, 66]. However, HER-2^+ patients with CNS metastases who are treated with trastuzumab appear to have a longer overall survival duration than those who are HER-2^- or those unselected for HER-2 status. This may reflect greater control of extracranial disease as a result of trastuzumab therapy [67].

Treatment with trastuzumab is associated with a higher risk for cardiomyopathy (left ventricular dysfunction and congestive heart failure), particularly when used in combination with paclitaxel or anthracyclines [68]. However, these cardiotoxic effects appear to be reversible once trastuzumab treatment is discontinued or if they are managed with appropriate medical therapy [69, 70]. The cellular mechanisms contributing to the cardiotoxicity observed with trastuzumab are still being explored. It is known that HER-2 plays an important role in cardiomyocyte development and function, and trastuzumab-induced inhibition of HER-2 signaling in cardiomyocytes may be a central mechanism underlying the observed cardiomyopathy [71]. However, the full explanation is likely to be more complex. Cardiotoxicity does not appear to be an issue with the TKI lapatinib, which inhibits both HER-1 and HER-2 [71]. Although cardiotoxicity is the primary safety concern with trastuzumab, potentially severe hypersensitivity reactions to infusion have also been reported [31].

In summary, trastuzumab is an effective treatment for patients with HER-2^+ disease, although its use is limited to this group (approximately 25%) [20]; accurate patient selection for treatment is important, using an appropriate method, such as immunohistochemistry or fluorescence in situ hybridization, to detect HER-2 overexpression. Additionally, not all HER-2^+ patients respond to treatment with trastuzumab, and the development of resistance is an issue. In the future, it may be possible to overcome resistance by combining trastuzumab with new therapies such as pertuzumab, by switching to an agent such as lapatinib that inhibits both HER-1 and HER-2 activity, or, if proven effective, the use of one of the pan-HER inhibitors currently in development. The efficacy that trastuzumab has demonstrated in the metastatic setting has provided the rationale for several studies investigating the use of trastuzumab plus chemotherapy as adjuvant treatment for patients with early-stage HER-2^+ breast cancer, a key area for development [72–77]. Cardiotoxicity remains a key safety concern for the use of trastuzumab, although in most patients this is reversible once trastuzumab is discontinued and/or appropriate medical treatment is given [68–70].

Intracellular Targeted Therapies: TKIs

Lapatinib. Lapatinib is approved in the U.S. (March 2007) and European Union (EU) (June 2008) for use (oral administration) in combination with capecitabine for the treatment of patients with advanced breast cancer or MBC whose tumors overexpress HER-2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab [78, 79].

In a pivotal phase III study that provided the basis for regulatory approval, a combination of lapatinib plus capecitabine led to a significantly longer median TTP than with capecitabine alone (8.4 months versus 4.4 months; $p < .001$) in patients with progressive, HER-2^+, locally advanced or MBC refractory to trastuzumab ($n = 324$) [80]. This was a notable finding, in that 97% of the patients had previously received treatment with trastuzumab and given that the data were similar to those reported in the phase III trastuzumab plus capecitabine trial in patients progressing on trastuzumab (TTP, 8.4 months versus 8.2 months) [43]. The incidences of adverse events (including those leading to treatment discontinuation) and symptomatic cardiac events were similar in both treatment groups [80].

Additional studies are ongoing to evaluate lapatinib in combination with trastuzumab, other chemotherapy agents, hormonal therapy, anti–vascular endothelial growth factor receptor (VEGF) agents, and as adjuvant therapy [59, 81, 82]. A recent phase III trial (EGF104900) showed a significantly longer PFS time with the combination of lapatinib and trastuzumab than with lapatinib alone (12 weeks versus 8.1 weeks; $p = .008$; hazard ratio [HR], 0.73) in patients with heavily pretreated, HER-2^+ MBC progressing on trastuzumab [59]. A summary of key lapatinib combination trials is presented in Table 3 [59, 80, 82–84].

Contrary to trastuzumab, lapatinib has activity against CNS metastases in patients with HER-2^+ breast cancer [85–87]. These data suggest that, as a small molecule TKI, it may be able to cross the blood–brain barrier to provide effective therapeutic concentrations in cerebrospinal fluid (unlike monoclonal antibodies such as trastuzumab).

Lapatinib appears to be associated with less cardiotoxicity than trastuzumab. An analysis of 3,689 patients treated with lapatinib in clinical trials reported a 1.6% incidence of cardiac events, with most events being asymptomatic and

Table 3. Summary of key lapatinib combination trials

Trial	Phase	Patient characteristics	Treatment regimen	Primary endpoint	ORR	PFS/TTP	Safety
[80]	III	HER-2$^+$, locally advanced breast cancer or MBC refractory to trastuzumab (n = 324)	L + X versus X	TTP	22% (L + X) versus 14% (X) (p = .09)	Median TTP, 8.4 mos (L + X) versus 4.4 mos (X) (p < .001)	Adverse events and cardiac events similar in both groups
EGF104900 [59]	III	Heavily pretreated MBC (n = 296)	L + T versus L	PFS	10.3% (L + T) versus 6.9% (L) (p = .46)	Median PFS, 12.0 wks (L + T) versus 8.1 wks (L) (p = .008; HR, 0.73; 27% lower risk for progression)	Manageable toxicity
VEG20007 [82, 83]	II	HER-2$^+$ MBC; cohort 1 (C1): P, 400 mg/day + L, 1,000 mg/day or L, 1,500 mg/day (n = 140); C2: P, 800 mg/day + L, 1,500 mg/day or L, 1,500 mg/day (n = 40)	L + PZ versus L	RR	C1: 12-wk RR, 36% (L + PZ) versus 22% (L); PD, no significant difference. C2: TBD	C1: 12-wk PFS, 84.1% (L + PZ) versus 63.2% (L) (p = .009)	Manageable toxicity; C1: diarrhea, rash, and nausea; C2: diarrhea, nausea, fatigue, hypertension, and rash
[84]	II	HER-2$^+$ advanced breast cancer or MBC (n = 52)	L + B	PFS	13%; PR, 7; CBR (CR + PR + SD ≥24 wks), 31%	12-wk PFS, 69%	Generally well tolerated; asymptomatic LVEF decline, n = 2, grade 2; LVEF dysfunction, n = 3, one grade 2 and one grade 1

Abbreviations: B, bevacizumab; CBR, clinical benefit rate; CR, complete response; G, grade; HER, human epidermal growth factor receptor; HR, hazard ratio; L, lapatinib; LVEF, left ventricular ejection fraction; MBC, metastatic breast cancer; ORR, objective response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; PZ, pazopanib; RR, response rate; SD, stable disease; T, trastuzumab; TBD, to be determined; TTP, time to progression; X, capecitabine.

reversible/nonprogressive [88]. A recent analysis from the Lapatinib Expanded Access Program, in which lapatinib was given in combination with capecitabine to 2,500 patients with advanced breast cancer, reported a 0.6% incidence of decreased left ventricular ejection fraction [89]. However, as lapatinib development is extended to include the treatment of patients with lower-risk primary breast cancer, it will be increasingly important to monitor cardiotoxic effects. The most common adverse effects associated with lapatinib treatment are gastrointestinal; lapatinib-related diarrhea generally occurs early in the course of treatment, is mild to moderate, and does not require treatment, although monitoring is important to identify patients who may need intervention [90].

Erlotinib and Gefitinib. In the U.S. and Europe, erlotinib monotherapy is currently approved for the treatment of patients with previously treated, locally advanced or metastatic non-small cell lung cancer (NSCLC) or locally advanced (U.S. only), unresectable (U.S. only) or metastatic pancreatic cancer [91]. Gefitinib monotherapy is currently approved in the U.S. for the continued treatment of NSCLC in patients who are benefiting or have benefited from treatment with gefitinib after failure of both platinum-based and docetaxel chemotherapies [92]; it has also now been approved in Europe for use in NSCLC patients with *EGFR* mutations, in all lines of therapy. Recent clinical studies have not demonstrated any significant clinical benefit for erlotinib or gefitinib either as single agents or in combination with other agents in MBC [93–99]. Given their lack of activity as monotherapy in MBC, studies continue to investigate the efficacy of erlotinib and gefitinib in combination with other targeted therapies, chemotherapy, or hormonal agents; however, tolerability issues may limit this approach.

Neratinib. Neratinib (HKI-272) is an orally administered, irreversible, pan-erbB kinase inhibitor [100]. The observation that some patients with chronic myelogenous leukemia were developing resistance to the TKI imatinib led to the development of neratinib. In preclinical models, neratinib has been shown to have promising antiproliferative activity in both HER-2–dependent cell lines and tumor xenografts. Clinical development has seen trials conducted in patients with NSCLC and in patients with breast cancer. In a phase I dose-escalation study in patients with solid tumors, the maximum-tolerated dose was determined to be 320 mg/day and the 240-mg/day dose was chosen for use in phase II studies. A total of 25 patients in that study had MBC. Of these, eight (32%) had a PR and one experienced SD for >24 weeks. Importantly, all responders were heavily pretreated, having received prior trastuzumab, anthracycline,

and taxane therapy [101]. Phase I/II data have confirmed that neratinib has antitumor activity in patients with HER-2^+ MBC, either as a single agent in trastuzumab-refractory patients or in combination with trastuzumab, and the safety profile of this agent has been manageable [102, 103]. Phase III trials that are ongoing include a study of single-agent neratinib in trastuzumab-pretreated patients with early breast cancer, a study of neratinib versus lapatinib and capecitabine in trastuzumab-pretreated MBC patients, and a study of neratinib plus paclitaxel versus trastuzumab plus paclitaxel as first-line therapy for patients with MBC [104].

In summary, experience with agents targeting the HER family shows that agents such as trastuzumab, lapatinib, and neratinib are clinically active in MBC and are generally well tolerated. However, evidence is increasing that agents targeting HER-1 alone are not associated with clinical benefit in the MBC setting. Accurate patient selection based on HER-2 overexpression is essential for trastuzumab-based treatment and is likely to be important for other agents in this class. However, identifying suitable patients may prove more difficult for TKIs, because receptor overexpression alone does not seem to predict response to treatment [13]. HER-targeted agents may need to be used in combination with chemotherapy to provide clinically relevant activity, according to classical ORR criteria. Targeting HER-2 is associated with cardiac toxicity, which is an especially important consideration in the adjuvant setting and when combining anti–HER-2 agents with cardiotoxic chemotherapeutic drugs. Targeting HER-1 in combination with HER-2, as with the TKIs lapatinib and neratinib, appears to reduce the risk for cardiotoxicity, although the exact mechanisms underlying this observation remain unclear.

Targeting Downstream Effector Molecules

Targeting HER receptors with extracellular monoclonal antibodies and intracellular TKIs has shown promising clinical activity. There is, however, a need for better treatment of MBC patients because many of these current therapies are restricted to a subset of the MBC patient population. Targeting cellular signaling pathways, such as the MAPK and PI3K pathways, downstream of HER receptors may be an attractive avenue for novel treatments. Additionally, there is some evidence that targeting heat shock proteins (Hsps) and the apoptotic pathway may be viable options for future therapeutic strategies in MBC. Recent developments in this field are briefly discussed in the following sections.

MAPK and PI3K Signaling Pathway Overview

The MAPK pathway, also termed the extracellular signal-regulated kinase (ERK) pathway, contains downstream effectors of the HER family and other tyrosine kinases, and is a central part of the signaling networks that control fundamental cellular processes, including cell proliferation, differentiation, and survival [105] (Fig. 1). The PI3K pathway also plays a central role in numerous cellular signaling pathways, and has been linked to a range of processes involved in tumor development, including cell proliferation, cell growth, cell motility, cell survival, and angiogenesis [24] (Fig. 1).

Targeted Therapies Directed at the MAPK and PI3K Signaling Pathways

The farnesyl transferase inhibitor tipifarnib (R115777) was evaluated in phase III trials for the treatment of breast cancer, although further development has now been terminated [106–108]. AZD6244 (ARRY-142886), an inhibitor of the enzyme MEK, a component of the MAPK pathway, is currently in phase I clinical studies in several cancer types, including breast cancer. Therapies targeting the PI3K pathway include perifosine (KRX-0401), which inhibits Akt phosphorylation [109], and the rapamycin analogs that target mammalian target of rapamycin, such as temsirolimus (CCl-779) [110–112] and everolimus (RAD001) [46, 47, 113, 114]. Recent data from two phase I trials suggest that everolimus can help overcome resistance to trastuzumab in women with HER-2^+ MBC. Everolimus plus trastuzumab and weekly paclitaxel was shown to slow tumor growth in 77% of patients, and the combination of everolimus with trastuzumab and vinorelbine halted tumor growth in 62% of patients [46, 47]. Although early indications suggest that targeting components of the PI3K pathway may have some activity in the treatment of MBC, additional data, including an understanding of combinations and patient selection, are required.

Apoptosis Signaling Pathway Overview

Apoptosis, the process of programmed cell death, is governed by complex, gene-directed pathways [115–117]. Dysregulation of apoptosis plays a key role in tumorigenesis and can allow tumor cells to become resistant to anticancer treatments [116, 117]. Rationale for targeting apoptosis in the treatment of breast cancer includes the overexpression of the Bcl-2 protein in 40%–80% of human breast tumors, which is associated with both resistance to chemotherapy [118] and a better prognosis after chemotherapy [119]. Additionally, the association of Bcl-2 with ER and/or PgR, loss of expression of the gene for the pro-apoptotic protein Bax, and differential expression of tumor necrosis factor–related apoptosis-inducing ligand-receptor 2 have all been correlated with prognosis in breast cancer patients [118–121].

Targeted Therapies Directed at the Apoptotic Pathway

Anticancer agents targeting the components of apoptotic pathways are in the early stages of development, and no agent specifically targeting apoptosis has yet been approved for use in cancer treatment. A range of approaches is being tested, including antisense DNA oligonucleotides and antibody and small molecule inhibitors of the components of apoptotic pathways. Few clinical data are currently available in breast cancer; however, preclinical studies show that such agents do have anticancer activity, suggesting that this may be a promising approach, particularly when used in combination with chemotherapy.

Hsp Signaling Pathway Overview

Hsp-90 acts as a regulator of the HER family by functioning as a chaperone protein, binding to and maintaining client molecules in their active conformation [122]. Hsp-90 is overexpressed two- to tenfold in human tumor cells [122]. Although Hsp-90 is associated with many cellular pathways and effectors, both HER-1 and HER-2 require chaperoning by Hsp-90 for their stability [122], and Hsp-90 is able to disrupt the ability of HER-2 to form signaling heterodimers on ligand binding [123].

Targeted Therapies Directed at the Hsp-90 Apoptotic Pathway

A range of Hsp-90 inhibitors has been developed and evaluated in clinical trials for the treatment of breast cancer, including tanespimycin (KOS-953, 17-AAG in Cremophor). Recent data from a phase II study of tanespimycin in combination with trastuzumab as second-line therapy in patients with HER-2^+ MBC showed evidence of good antitumor activity (CBR [PR + SD $\geq$4 months], 63%), and the combination was very well tolerated [44]. Further studies are needed to confirm the effectiveness and safety profile of current Hsp-90 inhibitors.

Targeting the Angiogenesis Pathway

The process of angiogenesis (the formation of new blood vessels from a pre-existing vascular bed) is complex and dynamic, and it is regulated by a range of pro- and antiangiogenic molecules [124]. The VEGF and platelet-derived growth factor (PDGF) families of proteins and their receptors (VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, and PDGFR-β) appear central to the process [124]. Activation of VEGFRs and PDGFRs initiates signaling that results in numerous cellular responses, including survival, mitogenesis, migration, proliferation, and differentiation [124, 125]. Activation of the VEGF pathway also increases vascular permeability and the movement of endothelial progenitor cells from the bone marrow into the peripheral circulation [124].

Primary breast tumors express a variety of different angiogenic factors, with VEGF being the most abundant. High VEGF expression appears to be correlated with poor prognosis and response [126]. Levels of VEGF in breast cancer tumors are a prognostic factor for relapse-free and overall survival in patients with both lymph node–negative and lymph node–positive disease [127, 128], and they predict response to both tamoxifen and chemotherapy in advanced disease [129]. Similarly, a proportion of invasive breast cancers that overexpress PDGFR-α have been associated with greater biological aggressiveness and a higher likelihood of lymph node metastasis [130].

It is increasingly being accepted that tumor cell proliferation alone is insufficient to result in a substantial tumor mass. Angiogenesis is essential for tumors to develop into detectable localized masses, and for metastasis to occur [131, 132]. Given their central roles in tumor angiogenesis and growth, the VEGF and PDGF signaling pathways are key targets for breast cancer therapy. However, with considerable redundancy in angiogenic signaling pathways, the inhibition of more than one receptor is likely necessary to block angiogenesis. It has been hypothesized that anti-VEGF agents may prevent the development of new tumor vasculature and induce normalization of existing, inefficient tumor vasculature (resulting from overexpression of VEGF) [133]. These agents, then, may allow better delivery of cytotoxic therapies to the tumor, suggesting a potential role for anti-VEGF therapy in conjunction with chemotherapy [133, 134].

Targeted Therapies Directed at Angiogenesis

Several therapies targeting angiogenesis are in development for breast cancer. These include monoclonal antibodies that act extracellularly by binding to receptors or their ligands, such as bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA), and TKIs that act intracellularly, such as sunitinib (Sutent®; Pfizer, Inc., New York) (Table 1). Bevacizumab is an anti-VEGF humanized monoclonal antibody administered as an i.v. infusion. It acts by binding to all VEGF isoforms, thus removing VEGF from the circulation and preventing activation of VEGFRs [135]. Bevacizumab is currently approved for use in combination with paclitaxel as first-line treatment for patients with MBC [136].

A number of single-agent TKIs with multiple molecular targets have been developed as an alternative to combining multiple agents. These were developed based on previous studies showing that combining agents that target different pathways may have synergistic activity and delay or reverse resistance [59, 137–139]. A range of oral, antiangiogenic TKIs with multiple targets is currently in development for MBC. These include sunitinib, pazopanib, sorafenib (Nexavar®; Bayer Pharmaceuticals Corporation, West Haven,

Table 4. Summary of key bevacizumab trials

Trial	Phase	Patient characteristics	Treatment regimen	Primary endpoint	ORR	PFS/TTP	OS	Safety
E2100 [151]	III	MBC, previously untreated (n = 722)	B + PX versus PX	PFS	ORR, 36.9% (B + PX) versus 21.2% (PX) ($p < .001$)	PFS, 11.8 mos (B + PX) versus 5.9 mos (PX) ($p < .001$)	OS, 26.7 mos (B + PX) versus 25.2 mos (PX) ($p = .16$)	Grade 3 or 4 hypertension was more frequent in B + PX arm
AVADO [152]	III	MBC, previously untreated (n = 736)	B (low dose + high dose) + D versus D	PFS	ORR, 55% (B + D low) versus 63% (B + D high) versus 44% (D)	PFS, 8.7 mos (B + D low) versus 8.8 mos (B + D high) versus 8.0 mos (D)	OS, NS	No difference in grades 3–5 bleeding events
[150]	III	MBC patients with prior therapy with both an A and a T (n = 462)	B + X versus X alone	OS	ORR, 19.8% (B + X) versus 9.1% (X) ($p = .001$)	PFS, NS	OS, NS	Well tolerated, more grade 3 or 4 hypertension with B + X than with X
[153]	III	MBC (or locally recurrent breast cancer), previously untreated patients	B + chemotherapy (X or T or A) versus placebo + chemotherapy (X or T or A)	PFS	ORR, 35.4% (B + X) versus 23.6% (placebo + X) ($p = .0097$); ORR 51.3% (B + T/A) versus 37.9% (placebo + T/A) ($p = .0054$)	PFS, 8.6 mos (B + X) versus 5.7 mos (placebo + X) ($p = .0002$); PFS 9.2 mos (B + T/A) versus 8.0 mos (placebo + T/A) ($p < .0001$)	OS, 29.0 mos (B + X) versus 21.2 mos (placebo + X) ($p = .27$); OS 25.2 mos (B + T/A) versus 23.8 mos (placebo + T/A) ($p = .83$)	Safety consistent with prior studies. No new signals seen in either chemotherapy group

Abbreviations: A, anthracycline; B, bevacizumab; D, docetaxel; G, grade; MBC, metastatic breast cancer; NS, not significant; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PX, paclitaxel; T, taxane; TTP, time to progression; X, capecitabine.

CT), and axitinib. Sunitinib selectively inhibits several receptor tyrosine kinases (VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, Kit, REarranged during Transfection, FMS-like tyrosine kinase [FLT]-3, and colony-stimulating factor 1 receptor) [140–145]. It has antiangiogenic and antitumor activities and is approved multinationally for the treatment of advanced renal cell carcinoma (RCC) and for gastrointestinal stromal tumors after disease progression on or intolerance to imatinib mesylate therapy [146]. Pazopanib targets VEGFR, PDGFR, and Kit and is currently in development in a number of tumor types, including breast cancer. Although originally developed as a Raf inhibitor, sorafenib also inhibits the activity of VEGFR-2 and VEGFR-3, PDGFR-β, FLT-3, and Kit; thus, it may inhibit tumor growth both directly (through Raf and Kit) and indirectly, through inhibition of angiogenesis [147, 148]. In both the U.S. and Europe, sorafenib is currently indicated for the treatment of patients with advanced RCC and for the treatment of unresectable hepatocellular carcinoma [149]. Axitinib inhibits all known VEGFRs, in addition to PDGFR-β and the stem cell factor receptor Kit, and is currently being investigated in a range of tumor types, including breast cancer.

Extracellular Targeted Therapies: Monoclonal Antibodies

Bevacizumab. Phase III studies have investigated bevacizumab combined with chemotherapy (Table 4) [150–153]. In 2008, the U.S. Food and Drug Administration (FDA) approved bevacizumab in combination with paclitaxel for the first-line treatment of locally recurrent breast cancer or MBC [136]. This approval was controversial after the FDA's Oncologic Drugs Advisory Committee recommended that the phase III data (E2100) were insufficient to establish a favorable risk–benefit profile, with the efficacy data based on PFS rather than overall survival. The E2100 study reported a 52% lower risk for disease progression or death with bevacizumab in combination with paclitaxel than with paclitaxel alone and a doubling of the PFS time (paclitaxel alone, 5.8 months; combination, 11.3 months; HR, 0.48; $p < .0001$) [151].

Results from the phase III AVADO trial demonstrated that bevacizumab (7.5 mg/kg or 15 mg/kg once every 3 weeks) combined with docetaxel resulted in a significantly longer PFS time (the primary endpoint) than docetaxel alone (8.7 months versus 8.8 months versus 8.0 months for the low- and high-dose combination arms versus docetaxel alone) [152], although the treatment effect was not as robust as in the E2100 study. Based on the results from the AVADO trial, the existing EU label permitting the use of bevacizumab in combination with docetaxel was extended to allow bevacizumab to be combined with docetaxel, thus allowing additional patients access to bevacizumab treatment. Further analyses of the AVADO trial have revealed that treatment continuation with single-agent bevacizumab after discontinuation of docetaxel appears to delay disease progression [154], that there is no apparent correlation between the efficacy of bevacizumab plus docetaxel and hypertension or G-CSF use [155], and that the combination does not appear to be associated with a higher incidence of grade 3–5 bleeding events [156].

In summary, although bevacizumab has shown little activity as a single agent in MBC patients, combination therapy with chemotherapeutic agents has been associated with clinical activity in this patient population. This suggests that VEGF inhibition combined with chemotherapy is a promising treatment strategy in this setting. Further studies are under way to explore the use of bevacizumab with different chemotherapeutic regimens, hormonal treatments, and other targeted therapies (including lapatinib and trastuzumab) in patients with MBC. Additionally, trials of bevacizumab are ongoing in the adjuvant and neoadjuvant settings, and preliminary reports suggest that this approach may be feasible; however, there are concerns about hypertension and bleeding [157]. Indeed, hypertension, bleeding, and thrombosis remain potential safety concerns with a number of anti-VEGF therapies, and this area requires further study. Future trials should focus on identifying those patients who will derive the most benefit from bevacizumab-based regimens and how best to combine bevacizumab with other cancer therapies (which therapies should be combined and whether sequential or concurrent administration is most effective). Overall, growing clinical experience with agents targeting angiogenic processes, such as bevacizumab, has provided proof of concept for the use of these treatments in MBC patients.

Intracellular Targeted Therapies: TKIs

Sunitinib. Sunitinib has been shown to have antitumor activity in breast cancer preclinical studies, both as a single agent and in combination with chemotherapy [140, 143, 145]. Data from a phase II study of sunitinib monotherapy in patients with refractory MBC reported single-agent activity in heavily pretreated patients ($n = 64$), previously exposed to an anthracycline and a taxane [158]. The ORR was 11%, with a median TTP of 10 weeks, and toxicities were manageable [158]. Studies and case series evaluating sunitinib given in combination with taxane therapy for MBC have reported antitumor activity and a manageable and tolerable safety profile [159–161]. Briefly, sunitinib plus paclitaxel ($n = 20$) was generally well tolerated and showed preliminary evidence of clinical activity, with an ORR of 38.9% [159]. Sunitinib given sequentially with docetaxel ($n = 22$) also showed activity and a manageable safety profile [160, 161]. Of 18 evaluable patients in an exploratory study, 72.2% had PRs and 27.7% had SD [160]. In patients with HER-2^+ MBC, sunitinib combined with trastuzumab was tolerable and associated with a preliminary ORR of 24% ($n = 51$) [162]. A triple combination of sunitinib, trastuzumab, and docetaxel was also found to be clinically feasible in HER-2^+ MBC patients, with a preliminary ORR of 77.7% ($n = 18$) [163].

Phase III trials of sunitinib in combination with a variety of cytotoxic agents are under way in first- and second-line MBC therapy [164]. Although two phase III studies of sunitinib in the advanced disease setting (single-agent sunitinib in the first, second, and third lines of therapy and first-line sunitinib plus paclitaxel versus bevacizumab plus paclitaxel) have been stopped after preplanned interim analyses indicated that the primary endpoint would not be reached, other combination phase III studies are ongoing. These include SUN1099 (second- and third-line sunitinib plus capecitabine) and SUN1064 (first-line sunitinib plus docetaxel). Additionally, a phase II study is evaluating second-line sunitinib versus standard of care in previously treated advanced triple-negative breast cancer (SUN 1077) [104].

Pazopanib. Results of a phase II study (VEG20007) evaluating combination therapy with pazopanib and lapatinib versus lapatinib alone described superior activity with the combination of the two small molecule TKIs, pazopanib plus lapatinib versus lapatinib alone, in HER-2^+ patients ($n = 140$) (Table 3) [82]. At the interim analysis, the response rate (independent assessment) was higher for the combination (36%; $n = 32$) than for lapatinib monotherapy (22%; $n = 30$), whereas there was no significant difference in the rate of progressive disease [82]. This is the first phase II trial to demonstrate the clinical activity of TKIs (in the absence of chemotherapy) in the first-line treatment of MBC patients. However, because of the small sample size, heterogeneous population, and some missing efficacy data (15%–20% of patients in both groups), these can be considered preliminary data only.

Sorafenib. Sorafenib inhibited MAPK activity in breast cancer cell lines expressing mutations of *K-Ras* or *B-Raf*, and showed antitumor and antiangiogenic activity in a human breast cancer xenograft model [148]. Data from a phase II study in patients with MBC ($n = 23$) previously exposed to an anthracycline and/or a taxane showed no significant clinical activity with sorafenib (6-month OS rate, 81%; 2-month PFS rate, 53%; 4-month PFS rate, 24%; 6-month PFS rate, 6%) [165]. Single-agent treatment was well tolerated in that study; however, significant and sustained increases in blood pressure were reported in a study of sorafenib monotherapy in patients with metastatic solid tumors [165, 166]. Current data suggest little activity for sorafenib as a single agent in MBC patients; ongoing studies are exploring combination treatment with paclitaxel and with anastrozole in MBC.

Axitinib. In preclinical studies, axitinib was shown to selectively block VEGF-stimulated receptor phosphorylation

Table 5. Key trials of antiangiogenic TKIs in MBC patients, recruiting as of June 2009

Drug	Trial identifier	Phase	Patient characteristics	Expected *n* of patients enrolled	Treatment regimen	Line of therapy	Primary endpoint
Sunitinib	NCT00435409 (SUN 1099)	III	ABC	430	SU + X versus X	Second line	PFS
	NCT00393939 (SUN 1064)	III	ABC	550	SU + D versus D	First line	PFS
	NCT00246571 (SUN 1077)	II	ABC triple negative	200	SU versus standard of care chemotherapy	Second line	PFS
Pazopanib	NCT00509587	II	MBC	35	PZ versus PL	Second line	ORR
Sorafenib	NCT00632541	II	MBC	43	SB + B	Second line	PFS
	NCT00499525	IIB	MBC	180	SB + PX versus PX	First line	PFS
	NCT00622466	II	MBC	41	SB + PX	First line	ORR
	NCT00217399	I/II	MBC	50	SB + A	Second line	CBR
	NCT00722072	II	MBC	43	SB + F	Second line	PFS
	NCT00493636	II	MBC	220	SB versus PL	First or Second line	PFS

Abbreviations: A, anastrozole; ABC, advanced breast cancer; B, bevacizumab; CBR, clinical benefit rate; D, docetaxel; DR, duration of response; F, fulvestrant; MBC, metastatic breast cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PL, placebo; PX, paclitaxel; PZ, pazopanib; SB, sorafenib; SU, sunitinib; TKI, tyrosine kinase inhibitor; Triple negative, estrogen receptor negative, progesterone receptor negative, and human epidermal growth factor receptor 2 negative; X, capecitabine.

in vitro, resulting in the inhibition of endothelial cell proliferation and survival, and in a human breast cancer xenograft model it significantly inhibited tumor growth and disrupted tumor microvasculature as assessed by dynamic contrast-enhanced magnetic resonance imaging [167]. A randomized, double-blind phase II study of axitinib in combination with docetaxel versus docetaxel as first-line therapy for patients with MBC ($n = 168$) has shown that this regimen has promising antitumor activity [168]. In the overall patient population ($n = 168$), the TTP was 8.2 months for patients in the combination arm versus 7 months for patients treated with docetaxel alone ($p = .052$) and the ORR was 40% for patients in the combination arm versus 23% for those given docetaxel alone ($p = .038$). In a prior adjuvant chemotherapy subgroup ($n = 92$), the TTP was 9.0 months for axitinib plus docetaxel versus 6.3 months for docetaxel alone ($p = .012$) and the ORR was 45% for axitinib plus docetaxel versus 13% for docetaxel alone ($p = .003$) [168]. The treatment had an acceptable safety profile that was similar to that of other multitargeted agents [168]. Grade 3 or 4 adverse events that occurred at a higher rate with axitinib plus docetaxel than with docetaxel alone included febrile neutropenia, fatigue, stomatitis, diarrhea, and hypertension [168].

To date, the multitargeted TKIs discussed have not been validated in phase III trials in MBC patients, although there is preliminary evidence of clinical activity. Of the four agents described above, three (pazopanib, sunitinib, and axitinib) appear to have the most clinical activity to date. Based on experience with other targeted agents in breast cancer, and with these TKIs in other indications, combinations will hopefully show greater efficacy in the treatment of MBC. Current trials of multitargeted TKIs in MBC patients are summarized in Table 5.

Conclusions

In recent years, research efforts have focused on the signaling pathways involved in the growth and survival of breast cancer cells, leading to the development of a range of targeted agents with promising clinical activity. The encouraging success of trastuzumab, based on the identification of HER-2 as a molecular target, has provided the rationale for studying the array of targeted agents currently in clinical development for MBC. The various extracellular and intracellular targets of breast cancer therapies discussed in this review are presented in Fig. 3.

The lapatinib data have shown that other means of HER family targeting are effective, although the lack of success with erlotinib and gefitinib underlines the importance of careful evaluation of these agents. Ongoing research must define how and when to use trastuzumab and lapatinib in the various treatment lines for MBC. Numerous therapies that target intracellular signaling molecules are in development, and early evidence suggests that some of these agents (everolimus and tanespimycin) may have clinical utility in

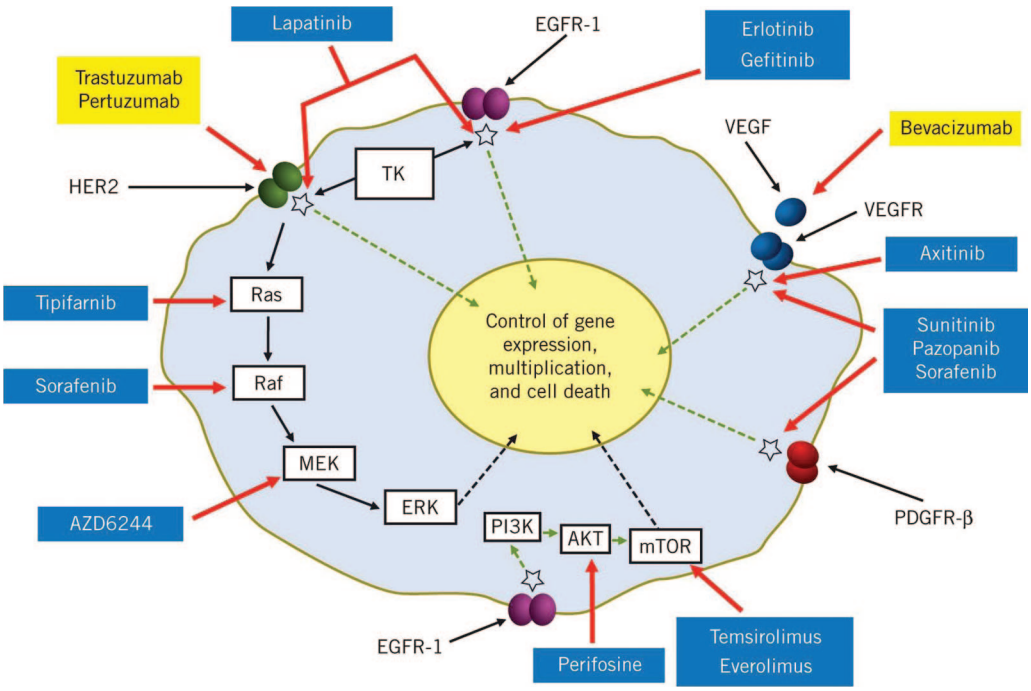

Figure 3. Extracellular and intracellular targets of therapies at various stages of development for breast cancer.

Abbreviations: EGFR, epidermal growth factor receptor; ERK, extracellular signal–related kinase kinase; HER-2, human epidermal growth factor receptor 2; MEK, mitogen-activated protein kinase/ERK kinase; mTOR, mammalian target of rapamycin; PDGFR-β, platelet-derived growth factor receptor β; PI3K, phosphatidylinositol 3-kinase; TK tyrosine kinase; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

MBC patients. Antiangiogenic drugs have also shown clinical benefit in this patient population, with the anti-VEGF antibody bevacizumab already licensed in combination with chemotherapy. Therapies targeting multiple receptors, including sunitinib and pazopanib, are also demonstrating clinical activity in early trials. It is anticipated that combining these treatments with other targeted agents and/or chemotherapy may offer an effective approach to the future treatment of MBC patients, but combinations of the targeted agents must be evaluated carefully for acceptable toxicity profiles.

Novel targeted therapies offer an attractive approach to the future treatment of MBC patients, with the prospect of individualized therapy based on the genetic expression profiles or clinical characteristics of individual patient's tumors. However, despite recent advances, there are many unanswered questions regarding the optimal treatment and long-term management of MBC patients with targeted agents. Future studies will need to address how best to incorporate these agents into existing treatment regimens, to identify those patient subgroups likely to derive most benefit from a given therapy, and to determine when and in which combinations targeted therapy should be administered.

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