Identifying Breast Cancer Druggable Oncogenic Alterations: Lessons Learned and Future Targeted Options

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Abstract Although the introduction of novel therapies and drug combinations has improved the prognosis of metastatic breast cancer, this disease remains incurable. It is therefore important to develop additional novel therapeutic strategies and agents. Increased understanding of the biology and the molecular alterations present in breast cancer is facilitating the design of targeted therapies directed to oncogenic proteins. Here, we review the signaling pathways and proteins that participate in breast cancer proliferation and survival, with special emphasis in those that are druggable. We will also comment on how the knowledge on the basic pathogenetic processes is translated into drug development strategies that are reaching the breast cancer clinic.

Breast cancer is one of the most common malignancies in the occidental world. In the United States, it is estimated that 178,480 patients will be diagnosed of breast cancer and 40,000 will die yearly due to this disease (1). Different treatment strategies have been proposed with the intention to improve survival, including diverse chemotherapy schedules and combinations or different targeted drugs. However, metastatic breast cancer (MBC) is still an incurable disease and most patients die due to this disease. In this context, the identification of molecular alterations that generate and sustain the tumorigenic process, together with the development of drugs that act on these alterations, may help to improve treatment efficacy and avoid resistance. From the clinical point of view, targeted treatment options for breast cancer patients include antiestrogen therapies for patients with estrogen receptor–positive tumors and anti-HER2 therapies for patients with HER2 overexpression. For the rest of the patients, and for those that progress to the previous therapies, chemotherapy is the standard approach. Taking into consideration the limited number of targeted therapies that had reached the clinic, and the poor prognosis of MBC, there is a clear therapeutic need for new active agents. This review highlights significant and druggable oncogenic alterations and describes the available compounds that are in clinical development. The review focuses on drugs that target specific proteins located (*a*) at the cell surface (membrane receptors), (*b*) in the cytosol (signaling intermediates), or (*c*) in the nucleus (proteins that control gene expression). We will also discuss recent progresses that focus on the targeting of breast cancer stem cells.

Agents That Act on Membrane Receptors

The regulation of cell number depends on the messages that the cell receives from the environment. These signals are usually transduced by cell surface receptors and regulate proliferation, survival, apoptotic cell death, or angiogenesis. In this section, we will review targeted therapies aimed at restricting tumor growth by acting on receptors that control these cellular processes.

The HER/ErbB receptors

The HER/ErbB receptors have been the focus of intense translational research, as two of them, the epidermal growth factor receptor (EGFR) and HER2, have been linked to several neoplastic diseases (2). The family of the EGFRs includes four members: ErbB1 or HER1, ErbB2 or HER2, ErbB3 or HER3, and ErbB4 or HER4 (3). These receptors share a similar structure, composed of an extracellular ligand binding domain, a transmembrane region, and an intracellular tyrosine kinase domain (Fig. 1). Several *in vitro* data have shown that the enzymatic activity of the kinase domain is critical for their biological function and transforming potential. This fact has focused research in the direction of understanding how the kinase activity of these receptors is regulated to design adequate treatments aimed at decreasing such activity. At present, it is quite clear that activation of these receptors depends on receptor-receptor interactions that facilitate tyrosine phosphorylation of one of the receptors by the other. Of course, this requires sufficient proximity of the receptors and a certain degree of temporal stability of these multireceptor complexes. Under physiologic conditions, activation occurs by ligand binding (3), which causes stabilization of receptor-receptor interactions. Following this process, the formation of homo-oligomers or hetero-oligomers between ErbB receptors provokes the transphosphorylation in tyrosine residues of the receptors and the recruitment of signaling proteins to these tyrosine

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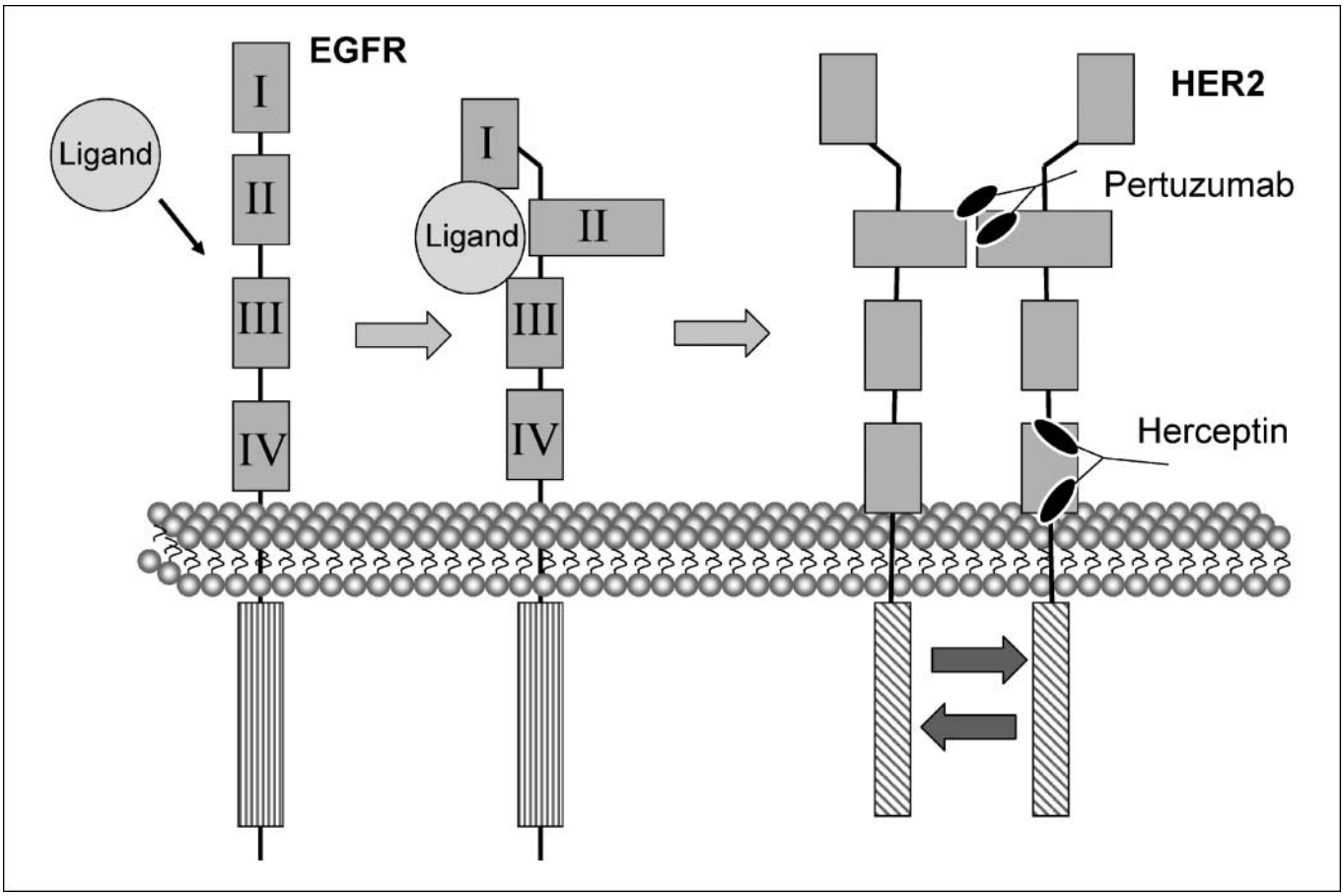

Fig. 1. ErbB receptor activation and anti-ErbB strategies. Ligand binds between domain I and II and provokes a conformational change with an elongation of domain II. This is the physiologic mechanism for all receptors but not for HER2, which has an open domain II in the absence of the ligand. Interaction between ErbB receptors is mediated by the union of two domain II. Pertuzumab binds to domain II and avoids the dimerization of both receptors. Herceptin binds to domain IV and provokes HER2 down-regulation.

phosphorylated sites, provoking the subsequent activation of intracellular signaling pathways. These pathways include the Ras/Raf/mitogen-activated protein kinase (MAPK) cascade, the phosphatidylinositol 3-kinase (PI3K)-AKT route, the signal transducers and activators of transcription, and phospholipase Cγ (4). Some of these proteins may translocate to the nucleus where they produce changes in gene expression that are responsible for the regulation of proliferation. In addition, recent findings have also suggested that HER2 as well as EGFR can act as transcription factors by a direct translocation to the nucleus (5).

Besides ligand binding, other mechanisms of activation frequently found in transformed cells include receptor overexpression or protein structural alterations such as mutations or truncations (6). Overexpression of HER2 has been found in approximately 20% to 30% of breast cancers and correlates with a more aggressive phenotype and a worse prognosis (7). EGFR mutations have been described in lung cancer, in which they also associate with response to EGFR antagonists (8). It is noteworthy that mutations in any ErbB receptor have not been described in breast cancer. Truncations of the extracellular NH_2-terminal region of the EGFR have been described in glioblastomas and correlate with responses to EGFR inhibitors (9). In a similar manner, truncations of the HER2 have been reported in breast cancer and have been associated with lack of response to Herceptin (10). The mechanisms proposed for the production of these forms include alternative translation or proteolytic cleavage (6).

Targeting HER receptors: monoclonal antibodies and small-molecule kinase inhibitors. As a consequence of the description of a link between HER2 overexpression and patient outcome in breast cancer (7), efforts were made to find agents that could decrease HER2 activity. Different strategies were designed, but only antibodies or small tyrosine kinase inhibitors have proven to be effective.

Herceptin as a prototype for the development of a targeted therapy. Herceptin is a monoclonal antibody (mAb) designed to target the extracellular domain of HER2. The mechanism of action of Herceptin is complex and has not been fully elucidated. Among the important actions of Herceptin are the removal of HER2 from the cell surface (11) and the induction of cycle arrest in G_1 through up-regulation of the cyclin-dependent kinase inhibitor p27 (12). As Herceptin binds HER2 on the COOH-terminal portion of domain IV (see below; Fig. 1), it also blocks activation of truncated HER2 produced by proteolytic cleavage of its extracellular region (13). One of the mechanisms that is critical in the action of Herceptin is related to antibody-dependent, cell-mediated cytotoxicity (14).

Herceptin is indicated in breast cancer tumors with HER2 overexpression. Although the criteria for Herceptin treatment in MBC include HER2 overexpression, it should be mentioned

that only one in three patients responds to Herceptin (15, 16). As a single agent, Cobleigh et al. (15) reported a response rate of 15% in 222 MBC patients previously treated with one or two chemotherapy regimens. A median duration of response of 9.1 months was observed. The tolerance was excellent, and cardiac dysfunction was observed in only 4.7% of the patients (15). Vogel et al. (16) evaluated single-agent Herceptin as a first-line treatment in 114 women with MBC. The overall objective response rate was 26% for the whole population. However, the response rate increased to 34% on considering the subgroup of patients with HER2 gene amplification measured by fluorescence *in situ* hybridization (16).

A larger trial was done by Slamon et al. (17) in 469 women with MBC. Patients were randomized to receive chemotherapy alone or chemotherapy plus Herceptin. Chemotherapy included standard doses of doxorubicin and cyclophosphamide with or without Herceptin. Patients who received anthracyclines in the adjuvant setting were randomized to receive paclitaxel alone or in combination with Herceptin. The addition of Herceptin to chemotherapy was associated with a higher objective response rate (50% versus 32%; $P < 0.001$), a longer time to disease progression (median, 7.4 versus 4.6 months; $P < 0.001$), and a longer survival (median survival, 25.1 versus 20.3 months; $P = 0.01$; ref. 17).

After these results, several chemotherapy agents have been tested in combination with Herceptin in the metastatic setting (18). In this context, and given the excellent activity of Herceptin in advanced breast cancer, several studies were planned in the adjuvant setting. The results of these studies have been reported recently, showing that the administration of Herceptin concomitant or sequential to chemotherapy produces an improvement in relapse-free survival and overall survival (reviewed in ref. 19).

Pertuzumab: the design of an anti-HER2 antibody based on the structure of HER2. The crystal structures of the extracellular domains of HER receptors have been solved in the last few years and include four distinct subdomains, named I, II, III, and IV. HER receptors share a common mechanism of activation that includes a receptor-receptor interaction that occurs between subdomain II. Physiologically, ligand-induced receptor activation occurs as a consequence of a first step that consists in ligand binding between subdomains I and III. This provokes a conformational change of subdomain II that extends a finger-like region, called the dimerization arm, which interacts with a homologous region of another HER receptor (Fig. 1). The potential participation of subdomain IV to ligand binding and activation is still unclear. It is important to indicate that Herceptin binds to domain IV, provoking the endocytosis and the subsequent down-regulation of the receptor. However, Herceptin does not prevent HER2 activation by soluble ligand (20). The fact that Herceptin does not prevent signaling by soluble ligand led to the development of strategies aimed at targeting subdomain II, with the purpose of preventing receptor-receptor oligomerization. Pertuzumab is a mAb that interacts with subdomain II (20). In contrast to Herceptin, pertuzumab is able to block the activation of HER receptors on ligand stimulation. Preclinical studies have shown its activity (20), and a phase I clinical trial has shown a low toxicity profile (20, 21). However, a phase II trial in MBC with low HER2 expression showed limited activity of this mAb (22). The reason argued for this lack of efficacy was the absence of selection of patients with HER2 overexpression. A recently reported study has shown that pertuzumab is active in combination with Herceptin after progression to this drug. In this study, a 21% response rate was observed in addition to a 50% stabilization rate (23). However, the clinical activity of pertuzumab as a single agent in HER2-overexpressing MBC patients needs to be explored further. At present, several ongoing clinical trials are testing its activity in patients with HER2-overexpressing tumors as well as in combination with other targeted drugs.

Small tyrosine kinase inhibitors. Inhibition of the tyrosine kinase activity with small cell-permeable compounds is one of the strategies used to interfere with the action of HER receptors. Gefitinib and erlotinib are two small tyrosine kinase inhibitors that bind with high affinity to the kinase domain of the EGFR. Several studies with these compounds showed disappointing results in MBC (reviewed in ref. 24). Likely, the residual effect of these inhibitors in breast cancer is due to the limited expression and biological function of EGFR in this pathology.

A novel class of tyrosine kinase inhibitors with a dual EGFR/HER2 activity have shown more promising results. This is the case of the dual inhibitor lapatinib. Burris et al. (25) did a phase I trial in heavily pretreated patients with ErbB1-expressing and/or HER2-overexpressing metastatic cancers. Lapatinib was well tolerated and signs of clinical activity were observed (25). Several clinical trials have explored the activity of lapatinib in combination with different chemotherapy regimens. A phase II trial with lapatinib monotherapy as a first-line treatment in metastatic patients with HER2 overexpression showed a 38% rate of partial responses (26). A phase III trial has shown that lapatinib in combination with capecitabine is active in HER2-overexpressing MBC patients that progressed after Herceptin-based therapy. In this study, the median time to progression was 8.4 months in the combination therapy group compared with 4.4 months in the monotherapy group (27). On the other hand, no increase in serious toxic effects or symptomatic cardiac events was observed in the combination arm. Recently, a randomized phase III study has shown that lapatinib in combination with paclitaxel as a first-line treatment is more active than paclitaxel alone in terms of response rates in an unselected HER2-overexpressing patient population. When this population was retrospectively studied for HER2 positivity, the combination of lapatinib with paclitaxel showed an increase in time to disease progression compared with paclitaxel alone (28). A specific breast cancer subtype in which lapatinib seems to be particularly active is inflammatory breast cancer. In this context, a phase II study has shown that the combination of lapatinib with paclitaxel is clinically active compared with chemotherapy historic controls (29).

Treatment combination options. Combination of anti-ErbB therapies has been studied in preclinical models and in clinical trials. Lapatinib in combination with Herceptin enhances apoptosis of breast cancer cells (30). In addition, preclinical studies have shown that lapatinib was active in breast cancer cells refractory to Herceptin. Following these results, a phase I clinical trial with the combination of lapatinib and Herceptin has been done (31). The reported results showed that the combination of both drugs is well tolerated and the regimen is clinically active. At present, different clinical trials are evaluating the administration of both agents in combination with other chemotherapy drugs, as well as the administration of lapatinib after progression to treatment with Herceptin. Other anti-HER

combination strategies to avoid alternative activating routes include the administration of Herceptin plus pertuzumab. Preclinical data have shown that this combination was active (32). Recently, a phase II clinical study has shown that pertuzumab can rescue from Herceptin resistance in metastatic HER2-overexpressing patients (23). However, this study must be taken with caution as long as the activity of pertuzumab as a single agent in the HER2-overexpressing population is pending to be defined. Further preclinical studies have explored the interaction of Herceptin with different chemotherapy drugs and have reported synergistic effects between the antibody and some of these drugs.

Targeting other receptor tyrosine kinases in breast cancer

The insulin-like growth factor-I receptor. The insulin-like growth factor-I receptor (IGF-IR) is a transmembrane tyrosine kinase receptor with a heterotetrameric structure that is commonly expressed in several normal tissues (33). Overexpression of IGF-IR has been associated to the development and maintenance of breast cancer (34). Activation of the IGF-IR provokes the stimulation of different signaling pathways, such as the PI3K or the Ras/Raf/MAPK pathways (33). During the last years, different strategies have been developed with the intention to inhibit this receptor, including small tyrosine kinase inhibitors or mAbs. Different studies have shown the activity of these compounds in several breast cancer models *in vitro* and *in vivo* (35). It is important to mention that breast cancer cells with IGF-IR overexpression are resistant to Herceptin (36). In a similar manner, breast cancer cells that acquire resistance to Herceptin express more IGF-IR than parental cells (35). Furthermore, inhibition of both transmembrane growth factor receptors increases the inhibition capability of each agent alone. This mechanism involves an increase in p27 and a subsequent cell cycle arrest (37). At present, anti–IGF-IR antibodies are being evaluated in the clinic. Recently, several phase I and II clinical trials have been reported using these mAbs alone or in combination with chemotherapy (see Table 1; ref. 38).

Targeting the vascular endothelial growth factor signaling system as an antiangiogenic strategy. Angiogenesis plays an essential role in breast cancer growth, invasion, and metastasis (39). Different signaling pathways have been involved in the production and maintenance of angiogenesis. The vascular endothelial growth factor (VEGF) family of proteins and receptors plays an important role in this process.

Bevacizumab is a recombinant humanized anti-VEGF antibody that binds with high affinity to all isoforms of VEGF-A. In preclinical models, the activity of bevacizumab has been shown alone or in combination with chemotherapies (40). A phase I/II clinical trial with bevacizumab alone in 75 patients with refractory MBC patients showed an objective response rate of 6.7% (41). Further confirmatory data have shown controversial results. A phase II trial showed no benefit in terms of progression-free survival and overall survival with the combination of bevacizumab plus capecitabine compared with capecitabine alone in MBC patients after treatment with doxorubicin and taxanes (42). However, the combination therapy significantly increased the response rates, 19.8% versus 9.1% (42). On the other hand, administration of bevacizumab in combination with paclitaxel as a first-line treatment in MBC showed an increase in disease-free survival (43). Recently, a single-arm phase II study has shown that the combination of capecitabine and bevacizumab is active and safe as a first-line treatment in MBC (44). At present, several clinical trials are exploring the role of bevacizumab with other drug combinations. Clearly, targeting the genesis and maintenance of vessels is an attractive idea but is likely to be insufficient unless accompanied by treatments that act on the tumoral cell.

Targeting apoptosis by acting on death receptors

Apoptosis is one of the natural mechanisms responsible for the control of cell number through programmed cell elimination. It is necessary in processes such as embryonic and tissue development, and tissue turnover (45). Apoptosis can be triggered by different stimuli, such as drug or irradiation treatments, stimulation of death receptors, or withdrawal of growth factors. Classically, two pathways are involved in apoptosis: the extrinsic pathway, activated by cell surface death receptors, and the intrinsic or mitochondrial pathway, which is usually activated by DNA damage and is controlled by the tumor suppressor gene *p53* (46). During the last few years, different strategies have been developed with the intention of targeting the extrinsic and intrinsic pathways. DR4 and DR5 are two death receptors involved in apoptosis activation (46). Recombinant human Apo2L/TRAIL is a protein that activates both receptors. Recombinant human Apo2/TRAIL has been studied in different preclinical models and has shown its ability to induce apoptosis without affecting normal cells (47). At present, this drug is being evaluated in patients with solid tumors in phase I clinical trials (48). Analogously, apomab, a mAb that acts on DR5, has shown antitumor activity in different tumor types, including breast cancer. As a consequence, clinical trials are ongoing, testing apomab in patients (49). Other antibodies have been developed against different apoptosis receptors, as is the case of mapatumumab that acts as an agonist of DR4. Recently, a phase I clinical trial has shown the safety of this drug, permitting the development of phase II studies (38).

Agents That Act on Cytosolic Events

The Ras/Raf/MAPK pathway

The Ras proteins are members of a large family of GTPases that play a crucial function in the transduction of signals from membrane tyrosine kinase receptors to the nucleus (Fig. 2; ref. 50). Downstream signaling activated by Ras-GTP involves the Raf-1 serine-threonine kinase pathway, which phosphorylates different kinases, finally provoking the activation and translocation of extracellular signal-regulated kinase 1/2 to the nucleus. In addition, Ras can activate the PI3K pathway. Contrary to other tumor types, such as pancreatic cancer, oncogenic Ras mutations are rare in breast cancer (50). Different drugs have been developed to target this intracellular signaling pathway, including farnesyltransferase inhibitors, Raf inhibitors, and MAPK/extracellular signal-regulated kinase inhibitors.

Farnesyltransferase inhibitors. Ras prenylation is required for its membrane localization and subsequent activation (50). Farnesyltransferase inhibitors were developed to inhibit this process and prevent Ras activation (50). However, farnesyltransferase inhibitors can also block the farnesylation of other proteins, confirming that these agents are not specific.

In MBC, a phase II trial with the oral farnesyltransferase inhibitor tipifarnib (R115777, Zarnestra) showed a modest

Table 1. Oncogenic pathways and selected druggable options

Target	Drug	Study
ErbB receptors	Antibodies	
	Pertuzumab (Omnitarg; Genentech)	Phase II
	Small tyrosine kinase inhibitors	
	Dual ErbB1-HER2	
	Lapatinib (GlaxoSmithKline)	Phase II-III
	EKB-569 (Wyeth)	
	HER2 CP-724714 (Pfizer)	Preclinical
IGF-IRs	Small tyrosine kinase inhibitors	
	NVP-AEW541 (Novartis)	Preclinical
	BMS-536924 (Bristol)	Preclinical
	Antibodies	Preclinical-phase I
	IMC-A12 (Imclone)	Phase I
	AMG 479 (Amgen)	Phase I
	CP 751,871 (Pfizer)	Phase I
PI3K/AKT pathway	PI3K inhibitors	Preclinical
	SF1126 (Semafore)	Phase I
	mTOR inhibitors	
	Everolimus (RAD001; Novartis)	Phase II
	Temsirolimus (CCI-779; Wyeth)	Phase II
	AP23573 (Ariad)	Phase II sarcomas
	AP23841 (Ariad)	Preclinical
Src inhibitors	Dasatinib (Bristol-Myers Squibb)	Phase II
	AZD0530 (AstraZeneca)	Phase II
	Bosutinib (SKI-606)	
HDAC inhibitors	SAHA (Merck)	Phase II
	CI-994	Preclinical
	LBH-589 (Novartis)	Preclinical
Proteasome pathway	Proteasome inhibitors	
	Velcade (Millenium)	Phase II
Chaperone inhibitors	17AAG (KOS-953)	Phase I-II
Angiogenesis	Anti-VEGF bevacizumab (Avastin; Roche)	Phase II-III
	VEGF-Trap	Preclinical
	Multityrosine kinase inhibitors	
	VEGFR2 (sunitinib, Sutent; Pfizer)	Phase II-III
	Sorafenib (Bayer)	Phase II
	VEGFR2-MET (XL880; Exelixis)	Phase I
	VEGFRs (vatalanib; Novartis)	
	Antibodies anti-VEGFRs	Preclinical

Abbreviations: mTOR, mammalian target of rapamycin; 17AAG, 17-allylamino-17-demethoxygeldanamycin; HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid.

activity with a low toxicity profile, consisting mainly in neuropathy and myelosuppression. The objective response rates ranged from 10% to 14%, with an additional 9% to 15% of patients with stable disease for 6 months or more (51). Different clinical trials are using farnesyltransferase inhibitors in combination with chemotherapy. This is the case of a study in which tipifarnib was administered in combination with doxorubicin and cyclophosphamide in patients with advanced and locally advanced breast cancer. The regimen was safe and clinically active. Pathologic complete response in the breast was seen in 7 of 21 (33%) patients, which is only slightly more active than the standard response observed for doxorubicin and cyclophosphamide (52).

Raf and MAPK/extracellular signal-regulated kinase inhibitors. Raf inhibitors are also in clinical development. Sorafenib (Bay 43-9006) is a small kinase inhibitor that blocks signaling proteins and receptors, including B-Raf, VEGF receptor (VEGFR) 2, VEGFR3, and c-Kit. Sorafenib is in clinical development in a wide range of solid tumors. For instance, in advanced renal cell carcinoma, sorafenib increased progression-free survival compared with placebo (53). In breast cancer, no data about the clinical activity of this compound have been reported, although different clinical trials are ongoing.

CI-1040 is an oral, selective MAPK/extracellular signal-regulated kinase kinase 1/2 small-molecule inhibitor. A phase II trial was done in patients with advanced breast, colorectal, non–small cell lung, and pancreatic cancer. A cohort of 14 MBC patients treated with two prior chemotherapy regimens was included in the study. However, no response rates were observed for all patient populations (54).

The PI3K/AKT/mammalian target of rapamycin pathway

The PI3K pathway is involved in the regulation of multiple cellular functions, including cell survival and proliferation (55). PI3K is a lipid kinase composed of a regulatory p85 subunit and a catalytic p110 subunit. Regulation of its activity depends on positive signals, usually triggered by activation of growth factor receptors, and negative regulation by the phosphatase PTEN. On activation, PI3K phosphorylates membrane phosphatidylinositols, which recruit several proteins with pleckstrin homology domains to the cell membrane, where they can interact with their downstream targets (56). During the last years,

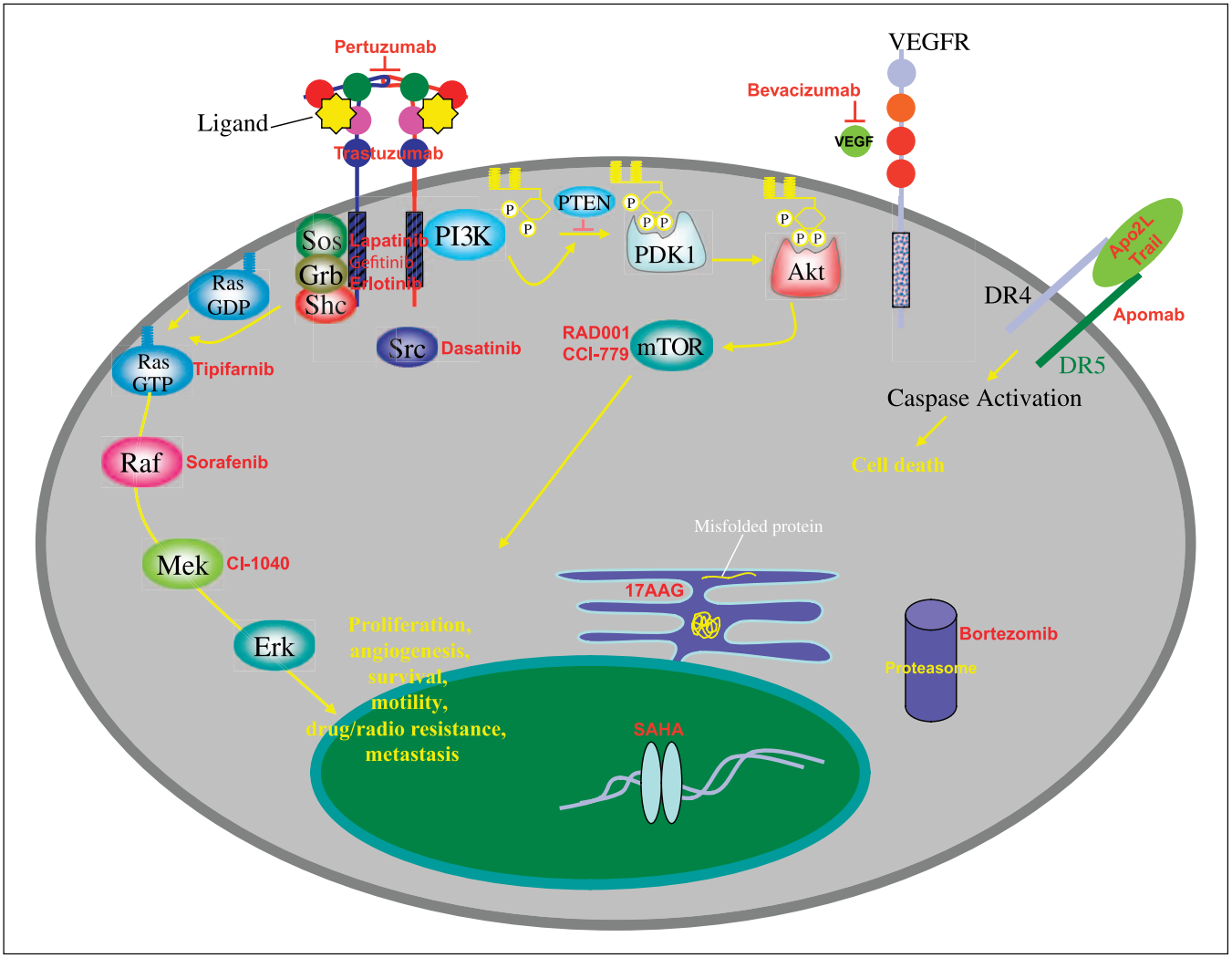

Fig. 2. Intracellular signaling pathways and targeted druggable options. mTOR, mammalian target of rapamycin; Mek, MAPK/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase; 17AAG, 17-allylamino-17-demethoxygeldanamycin.

a broad spectrum of PI3K inhibitors is in clinical development, and several of them are entering into phase I clinical trials (Table 1; ref. 55).

Downstream signaling effectors include AKT, which is involved in apoptosis, cell cycle regulation, protein synthesis, and glycogen metabolism. AKT also regulates the serine-threonine kinase mammalian target of rapamycin, which is implicated in the transcriptional and translational regulation of proteins involved in cell cycle control (57).

Recently, activated mutations of PI3K have been described in ~40% of primary breast cancer tumors, suggesting the importance of PI3K in breast cancer tumorigenesis (58). In a similar manner, the recent successful results of mammalian target of rapamycin inhibitors in preclinical models have suggested the importance of this pathway in breast cancer.

Temsirolimus (CCI-779) is a mammalian target of rapamycin inhibitor that is currently under clinical development. Several phase I trials showed the good toxicity profile of this compound. A phase II trial randomly assigned to receive 75 or 250 mg of temsirolimus weekly in heavily pretreated locally advanced or MBC patients. An objective response rate of 9.2% with a median time to progression of 12.0 weeks was observed (59). Recently, a phase III trial has compared temsirolimus plus letrozole versus letrozole alone in MBC. However, in this study, no improvement in progression-free survival was seen in the overall population (60).

Everolimus (RAD001) is another selective mammalian target of rapamycin inhibitor under clinical investigation. A phase I clinical trial showed a good toxicity profile (61). Analogous to temsirolimus, everolimus is being studied in combination with aromatase inhibitors.

Src inhibitors

C-Src belongs to the family of nonreceptor tyrosine kinases (62). Recently, preclinical data have shown that treatment with a small inhibitor of Src and Abl proteins, termed dasatinib, efficiently inhibits the proliferation of a panel of basal-like breast cancer cells (63). In addition, to explore this finding, a phase II clinical trial is ongoing, testing the efficacy of dasatinib in MBC patients with triple-negative tumors. In a similar fashion, other Src inhibitors are currently in clinical development as is the case of AZD0530 (64).

Targeting the chaperone machinery

Chaperones are proteins necessary for different cellular functions, including intracellular disposition, folding of proteins, or proteolytic turnover of regulators of survival and cell growth (65). Inhibition of HSP90 chaperone function induces proteasomal degradation of several proteins, such as hormone receptors, kinase proteins (receptor tyrosine or serine/threonine kinases), or AKT (65). Therefore, inhibition of HSP90 can potentially interrupt simultaneously several oncogenic proteins and pathways (65). Different drugs have been developed with the intention of targeting these proteins. Inhibition of HSP90 by binding to the ADP/ATP switch site is produced by ansamycins, such as 17-allylamino-17-demethoxygeldanamycin. Studies with this compound have shown activity in preclinical models alone and in combination with different chemotherapies (66). Based on preclinical data, most of clinical trials are focusing on specific tumor types in which a particular HSP90 client protein is known to play an important role, as is the case of HER2-overexpressing breast cancer (67). For instance, combination of 17-allylamino-17-demethoxygeldanamycin with Herceptin increases its inhibitory effect in breast cancer cells (68). Furthermore, a recent phase I trial with this combination showed a good toxicity profile with no unexpected toxicities. Indeed, in this study, a promising activity was observed in breast cancer patients with HER2 overexpression (69).

The ubiquitin-proteasome pathway

The ubiquitin-proteasome pathway is one of the cellular systems responsible for protein degradation. It is decisive for controlling different cellular functions, including signal transduction, transcriptional regulation, receptor function, and response to stress (70). Multiple ubiquitin molecules bind to the protein substrates that are subsequently degraded by the multicatalytic proteasome complex. Proteasome target proteins include p53, Bax, the cyclin-dependent kinase inhibitors p27 and p21, and IκB (70). Bortezomib is the only proteasome inhibitor that has been approved for the treatment of cancer patients with multiple myeloma. Preclinical data have shown the activity of this compound in different breast cancer cell lines (71). However, a phase II trial in MBC patients did not show any sign of efficacy (72). In this context, further studies have been planned to explore the role of bortezomib in combination with chemotherapy or targeted drugs, such as Herceptin.

Agents That Act on Nuclear Events: Targeting Epigenetics

Epigenetic is the term used to refer to the hereditable changes in the pattern of a gene that are not directly mediated by differences in the nucleotide sequence of DNA. The mechanisms involved in epigenetic regulation of gene expression include the acetylation of histone proteins or methylation of DNA in promoter regions of genes (73). Strategies to target epigenetics have been developed during the last few years. Among others, it is important to mention the role of the histone deacetylase inhibitors. These agents affect the expression of genes, such as the cell cycle kinase inhibitor p21 (74), cyclin D1, HER2, or thymidylate synthase. Suberoylanilide hydroxamic acid is a histone deacetylase inhibitor, which inhibits class I and II histone deacetylases. Administration of suberoylanilide hydroxamic acid in combination with docetaxel or Herceptin has shown a synergistic interaction in breast cancer cell lines through an increase in p21 and p27 levels as well as induction of apoptosis (75). In a similar manner, other histone deacetylase inhibitor, termed NVP-LAQ824, sensitizes cancer cells to Herceptin, provoking a down-regulation of HER2 (76).

Targeting Breast Cancer Stem Cells

During the last years, different studies have shown that a small proportion of cancer cells have the ability of self-renewal and may be able to reproduce the tumor when injected in nude mice. This population can be identified using different surface markers depending on the tumor type. In breast cancer, the $CD44^+$ and $CD24^-$ cells have been identified as the subpopulation of tumoral cells with the capability of self-renewal (77). The suggestion that this population could be implicated in chemoresistance, radioresistance, and tumor relapse has raised the importance of targeting these cells (78). In this context, an important effort has been recently made to identify the oncogenic alterations that could drive the initiation and maintenance of this cell subpopulation. These alterations can be produced at a genetic or epigenetic level and may affect different cellular functions, including proliferation, survival, differentiation, or angiogenesis (79). The transforming growth factor-β pathway has recently been implicated in breast cancer stem cell formation. In a recent report, a genetic study was done comparing $CD44^+$ with $CD44^-$ cells. In the $CD44^+$ cells, transforming growth factor-β was up-regulated and inhibition of this pathway produced a more epithelial phenotype (80). However, the function of transforming growth factor-β as an oncogene or as a tumor suppressor still limits the decision of targeting this route in breast cancer (81). More studies are needed to clarify the role of the transforming growth factor-β route in breast cancer stem cell biology.

Another interesting route that may have an important role in breast cancer stem cell biology is the Notch signaling pathway. This receptor is implicated in different functions, including differentiation, and acts mainly during organism development (82). Although Notch receptors have not been clearly linked to breast cancer stem cell formation, in other cancer types, its implication has clearly been shown (83). Activation of Notch involves cleavage of the protein that allows its translocation to the nucleus. Inhibitors of the γ secretase activity, responsible for this cleavage event, are in clinical development, and preliminary results in breast cancer have been disappointing (84).

The stem cell niche includes the vascular and stromal components, which are the two key pieces associated with the stem cell formation and activation. In other solid tumors, inhibition of angiogenesis in combination with chemotherapies was more active than chemotherapies alone to reduce the fraction of tumor stem-like cells (85). However, its implication in breast cancer is still pending to be defined. About the role of stroma in cancer stem cell progression, a recent report has shown the importance of mesenchymal stroma stem cells in the maintenance and formation of cancer cells and the incorporation of different potential oncogenic alterations that may favor metastatic dissemination (86).

Table 2. Future drug combinations depending on the genetic profile subtype

Subtype	Treatment strategy	Molecular alterations
Basal-like tumors	Chemotherapy + Platinum compounds	DNA repair mechanism
	Anti-EGFR therapies (cetuximab)	EGFR expression
	c-Kit expression	Anti-Src therapies (dasatinib)
	Multiple small tyrosine kinase inhibitors (sunitinib)	
Luminal tumors	Antiestrogen therapies	HR expression
	In combination with Herceptin in HER2-positive tumors	
HER2-positive tumors	Herceptin + chemotherapy	HER2 expression
	Herceptin + lapatinib	
	Herceptin + pertuzumab (after progression)	

Abbreviation: HR, hormonal receptor.

Future Options

The identification of druggable molecular alterations that rule the oncogenic transformation and progression has permitted the design of specific inhibitory strategies. This is the case of Herceptin and, more recently, the dual small tyrosine kinase inhibitor lapatinib. In most of the cases, the tumorigenic process involves different proteins and pathways that regulate tumor development. These multiple alterations often result in the lack of treatment efficacy of a specific inhibitor. In this context, there is a need for studying different drug combinations that target diverse signaling pathways. This idea has recently been supported by elegant studies in MBC from the Gupta group (87). Other hypothesis to explain the lack of treatment efficacy and tumor relapse is the presence of cancer stem cells and their association with therapeutic resistance, as well as other oncogenic alterations that are still pending to be defined.

Novel potential targets are being discovered based on cell biological and expression studies, as is the case of receptors that mediate apoptosis, growth factors, or their receptors. It is important to mention that breast cancer is a heterogeneous disease. At least, based on a genetic profile classification, four different cancer subtypes have been identified (88). For the luminal type and for the HER2-overexpressing type, treatment with antiestrogen therapy or with anti-HER2 therapies can be administered. However, for basal-like (triple negative) tumors, chemotherapy is the only available treatment. Data have shown that basal-like tumors overexpress EGFR and present alterations in the DNA repair machinery (89, 90). In this context, preclinical data show that the combination of anti-EGFR therapies with platinum compounds is extremely active in this particular situation. Based on this, ongoing clinical trials are exploring the role of DNA damage agents in combination with anti-EGFR therapies. Recently, a neoadjuvant phase II trial has shown a good clinical activity of cisplatin monotherapy in a triple-negative cohort of patients (91). Furthermore, in basal-like tumors, recent preclinical studies have shown that Src inhibition could be a good therapeutic option (Table 2; ref. 63).

From a clinical point of view, during the last few years, all novel drugs have been tested in MBC as a third- or fourth-line treatment. In this context, most of them have failed to show substantial antitumor activity. To overcome this problem, at present, novel drugs are given in combination with standard therapies as a first-line treatment in the advanced disease or in the neoadjuvant setting with the intention to permit a better evaluation of efficacy. Furthermore, the neoadjuvant approach allows pharmacodynamic studies, making different biopsies in the tumor, trying to identify any surrogated marker of treatment activity.

In conclusion, the identification of different oncogenic druggable alterations has permitted the development of directed targeted drugs to inhibit particular cellular proteins. However, the successful utility of these drugs in breast cancer patients must be shown in well-designed clinical trials.

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Identifying Breast Cancer Druggable Oncogenic Alterations: Lessons Learned and Future Targeted Options

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