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### Review Series

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# Targeted therapies for breast cancer

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**In recent years the description of well-defined molecular subtypes of breast cancer, together with the identification of the driving genetic alterations and signaling pathways, has led to the clinical development of a number of successful molecular targeted agents. This is best exemplified in the subset of HER2-amplified breast cancers, in which an increasing number of active agents are changing the natural history of this aggressive disease. Other targets are under exploration, and the clinical development of these agents will require a change from the current large, randomized trials in unselected patient populations to smaller trials in groups with a molecularly defined tumor type. In addition, combinatorial approaches that act on the secondary mutations and/or compensatory pathways in resistant tumors may markedly improve on the effects of targeted agents used alone.**

## Introduction

Breast cancer is a heterogeneous disease encompassing multiple subgroups with differing molecular signatures, prognoses, and responses to therapies (1). From the clinical view point, we can initially subdivide breast cancer into three major subtypes: tumors expressing estrogen receptors (ERs) and/or progesterone receptors (PRs) (commonly referred to as hormone receptor-positive [HR-positive] tumors), ERBB2-amplified (also known as human epidermal receptor 2–amplified [HER2-amplified]) breast cancer, and the remaining group commonly referred to as triple-negative breast cancer (TNBC) due to lack of expression of the ERs and PRs and normal or negative HER2 expression. This latter group itself encompasses a number of distinct entities with defined gene expression profiles and outcomes (1–3).

A greater understanding of the underlying biology of breast cancer has resulted in the identification of a number of molecular targets and development of novel therapeutics. Among them are tyrosine kinase inhibitors (TKIs) directed at a number of targets (HER1, HER2, HER3, IGF receptor [IGFR], C-MET, FGF receptor [FGFR]), inhibitors of intracellular signaling pathways (PI3K, AKT, mammalian target of rapamycin [mTOR], ERK), angiogenesis inhibitors, and agents that interfere with DNA repair. Some of these agents have shown remarkable activity and have already become part of the standard of care in patients with breast cancer (exemplified by the anti-HER2 agents trastuzumab and lapatinib). Others have shown clinical activity but are not yet approved for clinical practice. In this group are novel anti-HER2 agents as well as rapamycin analogs (“rapalogs,” or inhibitors of mTOR) and the poly(ADP-ribose) polymerase (PARP) inhibitors for BRCA-deficient tumors (4–6). The third (and clearly the largest) group of compounds are still in an early phase of development, but in some cases, indications of clinical responses have already been observed.

One of the challenges going forward will be the ability to match each patient with the right therapy. For example, without exception, anti-HER2 therapies are only effective in tumors with HER2 amplification/overexpression. Similarly, only BRCA mutant tumors display exquisite sensitivity to PARP inhibitors as single-agent therapy (5). Recent observations in other tumor types, such as the high responses observed in B-RAF mutant melanoma with B-RAF inhibitors or with ALK inhibitors in lung cancers harboring ALK mutations, provide support to the high rewards associated

with targeting therapies to tumors displaying certain mutations (7, 8). It is unclear whether we will find additional mutations that will correlate with a high level of drug sensitivity in breast cancer, but candidates include PI3K, AKT, and FGFR mutations as well as phosphatase and tensin homolog (PTEN) loss.

Other challenges to targeted therapies include acquired and primary resistance. Acquired resistance eventually develops in most patients in the advanced disease setting (9). Some mechanisms by which a tumor stops responding to a given therapy that it had initially responded to have been identified in HER2-positive tumors. These include loss of expression of the target as a result of continuous therapy (10), activation of mutations downstream from the target itself (11), and activation of additional mechanisms that promote cell proliferation (12). Primary resistance may occur due to lack of target dependency. In addition, it has been proposed recently that activation of compensatory pathways may rescue cells from the inhibitory effects of blocking just one target or pathway. For example, inhibition of the PI3K/AKT/mTOR pathway elicits compensatory activation of multiple survival routes including IGF1R and HER2, among others (13, 14). Combinatorial therapies that include clinical intervention at the level of these adaptive response mechanisms may improve outcomes, but distinct compensatory pathways are induced in a breast cancer cell type- and in a therapy-dependent context. Thus it is unlikely that a uniformly successful combinatorial approach to therapy will emerge.

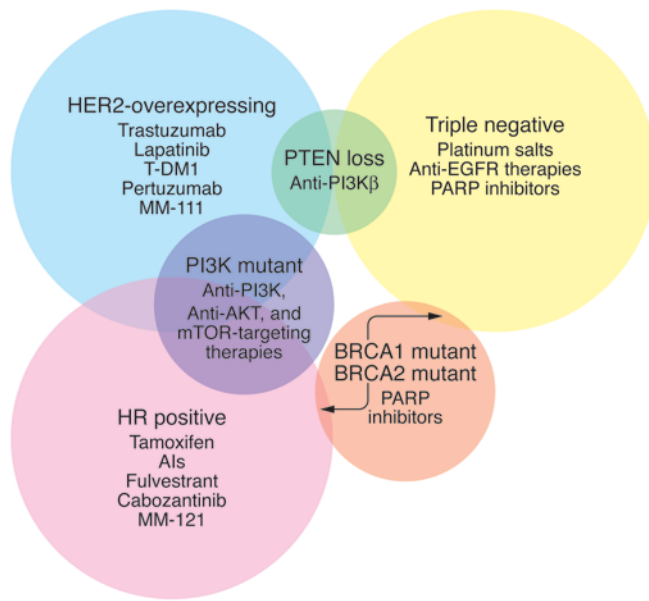
In this review we will highlight some of the most promising targeted agents in development and discuss considerations for the optimal design of clinical trials of targeted therapies in breast cancer. In order to simplify the presentation, we will use the sub-classification nomenclature currently used in the clinic. Targeted therapies currently available or in development for breast cancer subtypes are depicted in Figure 1.

## HR-positive breast cancer

ER- and PR-positive breast cancer has, for more than three decades, been the prime example of cancer amenable to targeted drug approaches. Estrogen-focused therapies remain pivotal to the treatment of this disease, with the ER modulator tamoxifen improving survival among women with early and advanced breast cancer and further improvements provided by aromatase inhibitors (AIs) and the ER-degrading agent fulvestrant (15–18). Their long-term efficacy, however, is limited by relapse of disease and development of resistance. Despite continuous expression of ER at relapse in either locally recurrent or secondary metastatic tumors,

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**Figure 1**  
Venn diagram of breast cancer subtypes and their overlapping molecular targets. Selected targeted treatment strategies are also depicted.

up to 50% of patients with HR-positive primary breast cancer who develop metastatic disease do not respond to first-line endocrine treatment (de novo resistance), and the remainder will eventually relapse despite an initial response (acquired resistance) (19).

The HER family of proteins comprises 4 receptors (EGFR/HER1 and HER2-4) activated by numerous extracellular ligands. Upon ligand binding the receptors dimerize, become phosphorylated, and transduce intracellular signals that regulate a variety of cellular processes including proliferation and survival. Resistance to therapy can occur as a result of cross-talk between the ER and HER themselves or between signaling pathways downstream of these receptors, such as PI3K/Akt/mTOR (ref. 20 and Figure 2). HER2 overexpression confers intrinsic or primary resistance to hormone-based therapy despite the presence of HRs. Within tumors that are both HER2 and ER positive, HER2 signaling is dominant, as demonstrated by the poor response of such tumors to endocrine therapy alone (21–24). This resistance can be partially overcome by combining anti-estrogen and anti-HER2 therapies. The addition of the anti-HER2 monoclonal antibody trastuzumab to an AI improved outcomes for patients with metastatic breast tumors that co-expressed both ER and HER2 (24). Likewise, in a large cohort of patients with known ER- and HER2-positive tumors, the addition of lapatinib, an anti-HER2 TKI, to an AI also significantly reduced the risk of progression (25). In contrast to HER2 and despite supportive preclinical data, observed clinical success with anti-HER1 inhibitors and endocrine therapy combinations has been limited (26–28).

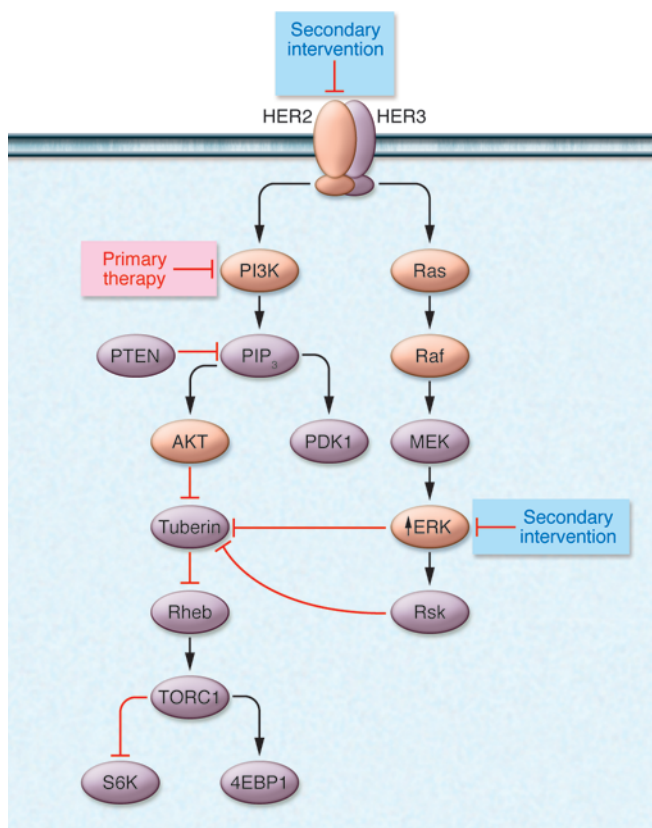
In addition to HER1 and HER2, there is growing interest in HER3 as a potential therapeutic target (29). Recently, HER3 and its physiologic ligand heregulin (HRG) have been implicated in the development of resistance to anti-estrogen therapies (30). There are now a number of anti-HER3 monoclonal antibodies in development, including MM-121, a fully humanized monoclonal antibody that binds to HER3 and prevents the HRG- and betacel-

lulin-induced phosphorylation of HER3 and also effectively inhibits the HER2/HER3 heterodimer (31). This compound is in Phase II studies, in combination with the nonsteroidal AI exemestane, in patients with advanced breast cancer that had previously progressed on endocrine therapies (32).

The PI3Ks phosphorylate the 3-hydroxyl group of phosphoinositides to activate second messenger molecules and set in motion a variety of physiological cellular metabolic and survival functions. Class IA PI3K molecules are heterodimers composed of a regulatory subunit (p85) and a catalytic subunit (p110), the  $\alpha$  isoform of which is widely mutated or amplified in human cancer (33). The PI3K signaling pathway is critical for the growth and survival of cancer cells in many human tumors including breast (34–36). For this reason, multiple PI3K inhibitors are currently in clinical development (33). These agents display variable specificity to the different PI3K subunits and an ability to inhibit other targets such as mTOR. It is likely, but yet unproven, that these agents will be of most benefit in breast tumors harboring a somatic mutation in *PIK3CA* (the gene encoding p110 $\alpha$ ) or in those with non-functioning or absent PTEN protein. Although *PIK3CA* mutations are found in all breast cancer subtypes (at a frequency of approximately 30%), they are most frequently identified in HR-positive or HER2-overexpressing tumors (36, 37). Although rarely mutated in breast cancer, diminished levels of PTEN expression through loss of heterozygosity and/or epigenetic silencing mechanisms are observed in up to 48% of breast tumors (38, 39). Interestingly, loss of PTEN has been shown to be more prevalent in triple-negative breast tumors and results in preferential activation of the PI3K $\beta$  subunit, an observation that suggests PI3K $\beta$ -specific inhibitors could be effective in this setting (40, 41). More advanced is the clinical development of rapalogs to inhibit mTOR. One of these agents, everolimus, has shown signs of improving the effects of AIs in a large presurgical study in patients with HR-positive breast cancer (42), and a randomized, placebo-controlled, Phase III study of everolimus in combination with exemestane is currently ongoing in the advanced disease setting (43).

As mentioned in the Introduction, inhibition of the PI3K/AKT/mTOR pathway elicits compensatory activation of multiple survival routes (13, 14). For example, it has been shown in tumors that inhibition of mTOR with rapalogs releases a negative feedback loop, resulting in activation of IGF1R signaling and ultimately phosphorylation of AKT (44). This activation may be prevented if IGF1R signaling is blocked with anti-IGF1R monoclonal antibodies. This finding led to a Phase I clinical study combining ridaforolimus (a rapalog) and dalotuzumab (an antibody targeted against IGF1R) that showed remarkable clinical activity in breast cancer (45). This combination is now being explored in a larger, Phase II study restricted to women with HR-positive metastatic breast cancer (46).

Almost a decade ago it was first suggested that anti-angiogenic strategies should be combined with drugs that target the proteins needed for cell motility and invasion, including hepatocyte growth factor (HGF) and C-MET (47), the rationale being that expression of these increases under hypoxic conditions and drives tumor cell survival and invasiveness even when anti-angiogenic agents are employed (48). Dual inhibition of MET and VEGFR2 would therefore be predicted to block major escape mechanisms used by tumors to overcome hypoxia. Cabozantinib (XL184; Exelixis) is a unique oral compound that inhibits multiple tyrosine kinases including MET and VEGFR2 (49). Preliminary data from an ongo-



**Figure 2**

The activation of compensatory pathways may contribute to the development of resistance to targeted therapies in HER2-positive breast cancer. Inhibition of PI3K results in the release of a negative feedback loop and activation of HER2 and, in turn, activation of ERK, a potentially detrimental effect. Strategies to prevent the compensatory pathway include intervention at different levels, i.e., at the receptor level or by blocking ERK.

ing Phase II, randomized discontinuation trial of cabozantinib suggest remarkable activity of this agent in visceral sites of solid tumors but particularly within bone metastases, perhaps because HGF is expressed by both tumor cells and bone stroma, and MET is subsequently highly activated in bone metastases (50). A Phase II study of this agent is planned in patients with metastatic HR-positive breast cancer to bone.

### HER2-overexpressing tumors

Less than a decade has passed since HER2 became an accepted therapeutic target in standard breast cancer practice. Trastuzumab in combination or sequence with cytotoxic chemotherapy transformed the prognosis of patients with HER2-overexpressing early breast cancer and is now routinely offered to this population mostly in combination with adjuvant chemotherapy (51, 52). Similarly, the dual HER1 and HER2 TKI lapatinib has clinical activity and is approved for the therapy of patients whose disease has progressed on trastuzumab (29). In addition to the approved agents, there are a number of novel strategies against HER2 that have shown activity in tumors that have progressed during treatment. One such approach has been the development of HER2-directed antibody-drug conjugates. Trastuzumab-DM1 (T-DM1) is an anti-HER2 antibody-drug

conjugate consisting of trastuzumab covalently bound via a linker to DM1, a derivative of the antimicrotubule chemotherapy maytansine. T-DM1 uses trastuzumab to specifically localize the highly active chemotherapy to HER2-positive tumor cells. Upon binding to HER2, T-DM1 is internalized and undergoes proteolytic degradation, resulting in release of maytansine only inside of HER2-overexpressing cells (53). In a Phase I study of T-DM1 in patients with heavily pretreated, advanced HER2-positive breast cancer that had progressed during prior trastuzumab therapy, the clinical benefit rate among patients treated at the recommended dose was 73%, including a number of objective responses (54). Single-agent activity was subsequently confirmed in a Phase II study among trastuzumab-resistant patients (55). Furthermore, preliminary results of a study of T-DM1 versus trastuzumab plus docetaxel in the first-line treatment of HER2-positive metastatic breast cancer patients suggest that T-DM1 provides similar efficacy to the combination with almost nonexistent side effects (56). It is now conceivable that we will develop systemic chemotherapy-free treatments for patients with HER2-amplified tumors.

Pertuzumab is a recombinant humanized monoclonal antibody directed against the dimerization domain II of HER2 that is required for ligand-dependent dimerization with HER3 (29). While trastuzumab prevents ligand-independent HER2 signaling, pertuzumab interferes with ligand-dependent HER3-mediated signaling. In preclinical models, combined anti-HER2 blockade with trastuzumab and pertuzumab shows a synergistic effect in HER2-positive tumors (57). The combination of pertuzumab and trastuzumab among patients with metastatic HER2-positive breast cancer who had experienced progression during prior trastuzumab therapy demonstrated a clinical benefit rate of 50% (4). Once again, a combination regimen that avoids the toxicity of systemic chemotherapy was highly active. The next logical question is whether T-DM1 and pertuzumab can be combined; thus an ongoing Phase III, randomized, three-arm, multicenter study will evaluate the efficacy and safety of T-DM1 plus pertuzumab or T-DM1 plus a placebo to pertuzumab, compared with the combination of trastuzumab plus taxane in patients with HER2-positive progressive or recurrent locally advanced or previously untreated metastatic breast cancer (58).

A similar approach is being studied with MM-111 (Merrimack Pharmaceuticals), a bispecific protein binding to HER2 and HER3 receptors, joined by a modified linker that inhibits HRG-induced activation of HER3 and AKT phosphorylation in dose-dependent manner. A Phase I/II trial of this compound in combination with trastuzumab is currently recruiting patients (59).

Resistance to anti-HER2 agents may occur as a result of aberrant activation of signalling pathways downstream of the receptor, such as the presence of activating *PI3K* mutations or loss of function of the phosphatase PTEN (refs. 11, 60, and Figure 2). In preclinical models, the addition of PI3K and/or mTOR inhibitors restores sensitivity to anti-HER2 agents (61). A recent Phase Ib study therefore combined the mTOR inhibitor everolimus with paclitaxel and trastuzumab in patients with HER2-overexpressing metastatic breast cancer pretreated with trastuzumab (62). Among the 27 patients evaluable for efficacy, the combination demonstrated encouraging antitumor activity with an overall response rate (ORR) of 44%. Overall disease was controlled for at least six months in 74% of those treated (62). HER2-targeting/PI3K inhibitor combinations are also in development. Another potential mechanism of resistance to HER2 monoclonal antibodies is the





generation of truncated forms of the HER2 receptor that lack the trastuzumab-binding domain; tumors that harbor the truncated receptor may be resistant to antibody-based therapies and preferentially sensitive to kinase inhibitors (63).

### TNBC

TNBC is an aggressive disease lacking a historical therapeutic target. In 2000, Perou et al. (2) first described distinct molecular subgroups of breast cancers based on variations in their gene expression profiles that correlated with prognosis; these subgroups included the basal epithelial-like group, an ERBB2-overexpressing group, a normal breast-like group and the ER-positive luminal A and B groups (1, 2). About 70% of breast tumors that are triple negative by immunohistochemistry cluster within the basal-like group of breast cancers on gene expression arrays (64). In addition to this overlap, a large majority of breast cancers arising in women with germline *BRCA1* mutations have the triple-negative phenotype and also cluster among the basal-like group (65). It is now well established that inhibiting the remaining DNA repair machinery within *BRCA*-deficient cancer cells using PARP inhibition results in synthetic lethality (66). To prove this biologic principle in breast cancer patients, 54 subjects with *BRCA1* or *BRCA2* mutations and advanced breast cancer were given the PARP inhibitor olaparib (AstraZeneca) (5). ORR was 41% among the 27 patients assigned to a 400-mg twice-daily dose without significant toxicity. These findings were complemented by the impressive clinical benefit, including a survival improvement, observed when another PARP inhibitor, iniparib (BSI-201; Sanofi), was combined with carboplatin and gemcitabine in a randomized Phase II trial among patients with TNBC (67). In late January 2011, the makers of iniparib (Sanofi) announced that the randomized Phase III trial evaluating iniparib in patients with metastatic TNBC (68) did not meet the prespecified criteria for significance for the coprimary endpoints of overall survival (OS) and progression-free survival (PFS), but the results of a prespecified analysis in patients who had received more than one prior line of chemotherapy for their metastatic disease demonstrated an improvement in OS and PFS, consistent with what was seen in the Phase II study (69). These results make a strong case to better define the tumor population that may respond to this agent as well to PARP inhibitors in general. To this end, complementary biomarker studies are being performed in concert with many of the ongoing PARP inhibitor clinical trials, including pharmacogenetic analyses, testing of blood and tumor tissue for PARP activity and expression, and analysis of DNA repair enzyme status (70–72).

HER1 is also a potential target in TNBC. The anti-EGFR monoclonal antibody cetuximab has been shown to have limited activity as single agent in TNBC (73) but does increase the antitumor activity of platinum salts. In one randomized Phase II study, cetuximab given in combination with cisplatin resulted in a doubling of the response rate and improvement in time to disease progression and survival when compared with cisplatin alone (74). However, the performance of cisplatin as a single agent was disappointing, and it is not clear that the combination of cisplatin and cetuximab would compare favorably to conventional therapy in this disease setting. Nevertheless, these results cannot be ignored, as they point out that a subpopulation of TNBC may be sensitive to EGFR inhibition.

### Targeting angiogenesis in breast cancer

The anti-VEGF-A monoclonal antibody bevacizumab was the first anti-angiogenic strategy to be rigorously evaluated in breast

cancer, although additional anti-angiogenic compounds are now being studied. The landmark E2100 study randomized patients to receive the mitosis inhibitor paclitaxel alone or with bevacizumab and demonstrated increased response rates (21.2% versus 36.9%, respectively) and significantly prolonged PFS (5.9 versus 11.8 months, respectively) with the addition of bevacizumab (75). Among the HR-negative group of patients enrolled on this study (the large majority of whom were also HER2 negative), the median PFS was 4.6 months with paclitaxel and 8.8 months with paclitaxel plus bevacizumab (75). Subsequent studies with other chemotherapies demonstrated similar improvements in PFS but failed to show an OS advantage (76, 77). One possible reason for this may be trial design, which in the case of the RIBBON-1 and AVADO studies allowed for crossover to bevacizumab-containing therapy at the time of progression and may have masked an OS advantage (76, 77). It has also been demonstrated that potent anti-angiogenic therapy over time can induce malignant progression of tumors due to increased invasiveness (78). Use of the bevacizumab has also been variably associated with an increased number of serious side effects, including stroke, wound healing complications and organ damage or failure (79). After careful review of the clinical data, the US FDA recommended that the breast cancer indication be removed from the bevacizumab label.

Sunitinib (Sutent; Pfizer) is a small-molecule multi-TKI that targets KIT, FLT3, RET, VEGFR2, and PDGFRB (80). In breast cancer, a single-agent Phase II study demonstrated clinical benefit in 16% of 64 heavily pretreated patients (81). However, two studies with sunitinib, either alone versus capecitabine or in combination with paclitaxel versus bevacizumab plus paclitaxel have been recently closed due to futility (82, 83). Sorafenib is another multikinase inhibitor targeting VEGFR1, VEGFR2, VEGFR3, PDGFRB, RAF, KIT, and FLT-3 (84). In a Phase II randomized trial in breast cancer patients, the addition of sorafenib to capecitabine significantly improved PFS compared with capecitabine alone (PFS 6.4 months versus 4.1 months) (85). The combination of capecitabine plus sorafenib is now being studied (versus capecitabine alone) in a large Phase III study (86).

The failure of bevacizumab to prolong OS has tempered expectations for the success of anti-angiogenic TKIs, but these compounds certainly have activity, and translational work to identify the subgroup of responsive patients will be critical. The lack of success of anti-angiogenic therapies to date in breast cancer may in part be explained by activation of additional pro-angiogenic switches upon blockade with bevacizumab, as has been shown in experimental systems (78).

### Design considerations for trials of targeted agents in breast cancer

There are a number of challenges that still need to be addressed, such as the identification of biomarkers of response and early markers of clinical benefit. The study of mechanisms of resistance, as mentioned above, is also critically important. A frequent mechanism of primary resistance is lack of dependency on the targeted gene or pathway, such as the lack of activity of PARP inhibitors in breast tumors with intact *BRCA* function. This provides a strong argument for the development of early biomarkers of response and for the development of novel agents only in the subpopulation of breast tumors that may be dependent on the targeted gene. Acquired resistance, on the other hand, can be the result of acquired mutations that “override” the mechanism of action of



the anticancer agent, such as mutations in the *PI3K* gene, downstream from HER2, which render cells insensitive to the effects of trastuzumab and lapatinib (87). This would require a combined therapeutic approach against the primary activating event (HER2 in this case) and the acquired mechanism of resistance (PI3K). There is an additional mechanism of resistance that relates to the activation of compensatory pathways that allow cells to “escape” the effects of therapeutic agents. Inhibition of certain molecular targets and/or pathways may result in activation of compensatory signaling pathways that prevent cell death. For example, AKT inhibition induces the expression and phosphorylation of multiple receptor tyrosine kinases (RTKs) (14). In a wide spectrum of tumor types, inhibition of AKT induces signaling via a conserved set of RTKs, including HER3, IGF1R, and insulin receptor; this may attenuate their antitumor activity. In this setting, combined inhibition of AKT and HER kinase activity is more effective than either alone (14). Similarly, inhibition of PI3K signaling results in activation of HER2 (13), which provides a strong rationale to block HER2 signaling in addition to PI3K.

Ongoing translational efforts should be focused on the development of standardized and validated biomarkers and functional imaging techniques that are able to indicate an early response (or lack thereof) to targeted agents in a variety of tumor types. These developments would ideally be tested in prospectively conducted, currently ongoing or completed clinical trials in which on-study tumor samples and other valuable materials including novel imaging approaches are carefully collected and annotated. Studies might also include patients with residual disease, in order to generate novel treatment approaches for this high-risk population. Such studies will require strong collaborations between clinical and laboratory researchers.

Once a biomarker has been identified, clinical trials of targeted agents may be performed within a specifically enriched patient population incorporating the predictive biomarker of clinical benefit. Study endpoints of clinical trials should therefore incorporate mechanistic effect measures on predefined markers and both tumor and stromal microenvironment. In addition, because compensatory pathways may be unknown and develop only during treatment with an experimental agent or combination of agents, efforts should be made to allow dynamic tuning of treatment selection within clinical studies. For example, trials would ideally incorporate a dynamic system of measurements of response or fail-

ure to the study agent, which would allow real-time inpatient change of treatment by either switching to another therapy or by adding a new agent. Such studies might explore collaboration with colleagues in functional imaging (e.g., PET, functional MRI) or use paired study tumor biopsies (baseline and on treatment) and circulating biomarkers in order to measure on-therapy effects. In breast cancer, the testing of new agents just prior to surgery (known as neo-adjuvant therapy) allows for monitoring of tumor response at the time of surgical resection. In comparison with the follow-up period of at least five years that is traditionally required to evaluate the efficacy of an adjuvant therapy, the neo-adjuvant setting enables the rapid assessment of tumor sensitivity within three to four months at most (42, 88–90). A further advantage is the easy availability of tumor tissue samples during the treatment period. A potential disadvantage of neo-adjuvant therapy in general is the loss of prognostic information provided by tumor size and nodal status at surgery and before adjuvant chemotherapy (91). These designs rely upon surrogate markers (such as changes in proliferation or apoptosis markers, or even absence of visible tumor at the time of surgery) that will then be correlated with endpoints of clinical benefit (such as time free of disease or improved OS).

## Conclusions

Current treatment options for breast cancer are moving toward nontoxic, potent targeted therapies that can be tailored to an individual patient's tumor. There are now targeted therapeutic options available for nearly all breast cancer subtypes, exploiting the differing drivers of carcinogenesis within these individual tumors. The continuing development, and indeed success, of these compounds will rely heavily on close collaborations between laboratory scientists and clinician researchers. The development of resistance to all of these therapies is an ongoing challenge and opportunity for learning. In concert with robust clinical trials of these agents, biomarkers of response or prediction of benefit to these interventions must be developed and validated. Just as cancer is a dynamic, adaptive process, so too must our clinical trial designs become innovative, flexible, and informative. In this way we will select the right patient, for the best drug or combinations of drugs, at the most effective time.

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