Targeted therapies offer a new approach to breast cancer treatment. Rather than eliminating both malignant and normal cells nonspecifically, these so-called “rational” therapies exploit second messenger proteins, ligands, and receptors that are known to be upregulated in neoplastic cells, or are implicated in cancer metastasis. This review will highlight a number of these targets and the mechanisms that have been targeted in drug design. We will also describe recently completed and currently ongoing clinical trials investigating targeted therapies and their potential to augment standard breast cancer therapy.

Incremental improvement in the efficacy of standard chemotherapy and adjuvant endocrine agents, as well as earlier detection of new breast tumors, have together altered breast cancer diagnosis and treatment during the past 25 years. Advances in therapy have led to rising 5-year survival rates and encouraging reduction in disease mortality.[1] However, traditional chemotherapy achieves its desired effects by targeting all rapidly dividing cells. Desired antitumor effects typically come at the expense of nonmalignant cells, specifically those in a high rate of turnover in the gastrointestinal tract and bone marrow. Therefore, the therapeutic index is significantly narrowed as efforts to obtain aggressive tumor eradication are weighed against achieving a tolerable side-effect profile. Greater understanding of the molecular biology of cancer has allowed novel additions to the chemotherapeutic armamentarium—“targeted” agents that exploit the characteristics unique to cancer cells for their eradication, rather than relying on the more universal “cytodestruction” of standard chemotherapy. Targeted therapies therefore maximize the therapeutic index by improving the efficacy of the anticancer treatment while also reducing toxicities to surrounding noncancerous host cells.

Targeting HER Family Receptors: Monoclonal Antibodies

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor important for normal cellular development, damage repair, and survival.[2] Each of the four receptors in the EGFR family-HER1, HER2/neu/ErbB-2, HER3, and HER4-has distinct ligand specificity, but all four possess a homologous transmembrane portion connected to an intracellular tyrosine kinase domain. Once bound to ligand, HER proteins must form either homodimers (eg, HER1/HER1) or heterodimers (eg, HER2/HER3) in order to activate intracellular phosphorylation. The only exception is HER2, which has no naturally existing ligand of its own, and is present only in low levels in normal human tissue as compared to HER1.[3] Instead, it acts as the preferred cofactor for ligand-bound HER1, HER3, and HER4 proteins, increasing the number of initiation stimuli for downstream signaling[4] (Figure 1). In tumor cells, EGFR is upregulated, resulting in increased activation of secondary messenger pathways and cell hyperproliferation.[5] Overamplification of the EGFR gene has been shown in a variety of human cancers including kidney, bladder, colon, pancreas, lung, rectum, and breast.[6] In addition, high levels of EGFR have been correlated with poor disease prognosis and lower survival among cancer patients.[7] Because of its widespread expression and relevant role in tumor development, the HER family receptors were some of the first targets to be selected for "rational" drug development.
The HER2/c-erbB2 gene is amplified in 25% to 30% of invasive breast tumors. [7] Preclinical experiments suggest that high levels of HER2 may forecast a poor prognosis in the same manner that large tumor size, high histopathologic grade, and lack of ER+ and PR+ expression are associated with negative outcomes in breast and ovarian cancers. Trastuzumab (Herceptin) is the first example of successful targeted therapy for breast cancer directed against the extracellular domain of HER2. [8] By blocking HER2, this recombinant humanized monoclonal antibody prevents kinased-mediated activation of the ras/raf/ MAPK and PI3K pathways, [9] therefore inhibiting the mechanisms that initiate tumor growth. Trastuzumab is currently used in the treatment of metastatic breast cancer, either alone [10] or in the presence of taxane chemotherapy. [11] Other phase II clinical trials have shown efficacy when trastuzumab was used with vinorelbine (Navelbine) [12] and gemcitabine (Gemzar). [13] Another recently completed phase III trial demonstrated that the combination of trastuzumab and paclitaxel with carboplatin (Paraplatin) resulted in improved response rate and time to progression as compared to trastuzumab and paclitaxel alone in women newly diagnosed with HER2-overexpressing breast cancers. [14] The successes of trastuzumab offer encouraging proof of principle for further targeted therapy development: by identifying unique characteristics of tumor cells, the tumor phenotype can be abrogated without major adverse effects on nonmalignant cells. The low but clinically relevant incidence of trastuzumab-associated cardiomyopathy is a notable exception. [15] Tumors with higher levels of HER2 by immunohistostchemistry (IHC) (ie, 3+ score) or with HER2 gene amplification using fluorescence in situ hybridization (FISH) have been shown to respond more convincingly to trastuzumab than those with less HER2 expression (IHC 2+ score). Initial phase II studies testing single-agent trastuzumab enrolled women with any HER2 expression at all, IHC 2+ or 3+ score. [16] While overall response to trastuzumab varied from 15% to 38%, the clinical benefit rate increased to 48% when only examining tumors that scored IHC 3+ for HER2 expression. In the absence of HER2 gene amplification, patients with tumors that exhibit IHC 2+ overexpression of HER2 do not appear to derive benefit from trastuzumab. [17] However, even among IHC 3+ tumors, barely 50% respond to trastuzumab. [18] Of those patients who benefit initially from treatment with trastuzumab, most progress again within 9 months. [17] A newer antibody, pertuzumab, has shown promising preclinical efficacy in breast cancer cell lines, and is currently being tested in phase I clinical trials. In contrast to trastuzumab's exclusive specificity for HER2, pertuzumab blocks all HER-mediated signal transduction by interfering with transmembrane receptor dimerization. [19]
Combined treatment with both antibodies is proposed to exhibit synergistic inhibition of EGFR signal transduction, while increasing the number of HER2-expressing tumors that will respond to anti-HER therapies.[19,20] In hopes of further improving the scope and efficacy of HER-targeted agents, a number of other anti-EGFR monoclonal antibodies are currently in development. For example, cetuximab (Erbitux) is a recombinant chimeric antibody directed against EGFR/HER1 receptor with an affinity greater than twice that of any of its natural ligands. Cetuximab has been shown to induce dimerization, internalization, and downregulation of the EGFR.[21] It has been shown to successfully inhibit tumor growth in many cancer lines including head and neck, colorectal, and pancreatic cancer.[22] While preclinical results in breast cancer cells were also promising, a small study of 13 women treated with paclitaxel and cetuximab did not demonstrate any promising antitumor activity and did encounter significant dermatologic toxicity (personal communication, A. Seidman, 2005).

In contrast to the aforementioned human-engineered antibodies, geldanamycin is a naturally occurring ansamycin antibiotic also under investigation as a potential HER2-targeted agent (Figure 2). Produced by the bacteria Streptomyces hygroscopicus during fermentation, geldanamycin has been shown to inhibit tumor growth in rodent fibroblasts by inhibiting intracellular tyrosine kinase phosphorylation.[23] Geldanamycin and its derivative, 17-N-allylamino-17-demethoxy geldanamycin (17-AAG), appear to block tyrosine kinase activity by inhibiting Hsp-90, a ubiquitous chaperone protein that stabilizes signaling proteins including EGFR.[24] Preclinical studies show that geldanamycin significantly inhibits tumor cell lines that overexpress HER-2, and in mouse xenografts, trastuzumab and 17-AAG demonstrated more superior tumor inhibition than either agent alone (unpublished data, D. Solit).
Currently, 17-AAG in combination with trastuzumab is being studied in a phase I/II trial at Memorial Sloan-Kettering Cancer Center (MSKCC) in patients with advanced breast cancer. This trial will examine the extent to which the mechanisms of action of the two drugs acting in concert can slow tumor growth, and will also determine the activity of 17-AAG alone in trastuzumab-resistant tumors.

**EGFR: Anti-EGFR Tyrosine Kinase Inhibitors** Tyrosine kinase inhibitors (TKIs) are the second main strategy for targeting HER-mediated signaling. These small-molecule inhibitors are modeled after imatinib mesylate (Gleevec), a well-validated TKI directed against the \( \text{bcr-abl} \) translocation that drives the development of 95% of chronic myeloid leukemias. By inhibiting the constitutive tyrosine kinase activity of this oncogene, imatinib therapy alone is sufficient to stop the malignant transformation of cells in chronic myeloid leukemia.[25] Likewise, a TKI bound to the intracellular ATP-binding pocket of the EGFR disrupts downstream phosphorylation and signaling pathways in solid tumor cell lines. By design, anti-EGFR TKIs offer potential advantages over anti-EGFR antibodies, as they are several-fold smaller (~400 Daltons, as compared to ~150,000-Dalton monoclonal antibodies) and therefore provide improved tumor penetration. In addition, they are administered orally rather than by intravenous infusion.[26] However, TKIs are less specific for malignant cells, and can cause mild to moderate toxicities including skin rash and diarrhea, as well as edema and headaches.[27]
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Anti-EGFR TKIs

have been moderately successful in achieving significant clinical response among patients with solid
tumors. Gefitinib (Iressa) is a reversible TKI that has demonstrated safety and tolerability in
dose-escalation studies of a variety of solid tumors,[28-30] including preclinical breast cancer tumor
models.[31] Large multicenter phase II trials confirmed antitumor activity in patients with advanced
non-small-cell lung cancer (NSCLC),[32,33] motivating the US Food and Drug Administration (FDA) to
approve gefitinib as third-line treatment for patients with NSCLC following failure of both docetaxel
(Taxotere)- and platinum-based therapies. Further studies by Lynch et al demonstrated that patients
with particular mutations within the EGFR gene experience a more rapid, dramatic response when
taking gefitinib, suggesting a more specific niche for TKIs that may improve their efficacy in treating
patients with NSCLC.[34] Emerging understanding of the biology underlying hormone-dependent
breast cancers demonstrates crucial "cross-talk" between EGFR-mediated and estrogen receptor
pathways, suggesting a possible role for gefitinib in the treatment of breast cancer as well. Shou et
al found that while breast cancer cells overexpressing HER2 seemed resistant to tamoxifen's
ER-inhibitory effects, using gefitinib in addition to tamoxifen enhanced antitumor effects, presumably
by inhibiting HER2-mediated EGFR phosphorylation and cross-activation of ER signaling
pathways.[35] Three recent phase II studies in women with metastatic breast cancer demonstrated
very slight clinical response or disease stabilization with gefitinib monotherapy. For example, Albain
et al reported a partial response in 1 of 63 women enrolled in the trial, and stable disease in 8
others. However, 24% (15 patients) continued to receive treatment after the required 2 months for
participation regardless of outcome, and 5 of 12 patients reported improvement in bone pain,[36]
offering some encouragement for further investigation of its use for breast cancer. The recent FDA
approval of erlotinib (Tarceva) a second anti-EGFR TKI, for the treatment of refractory stage IIIB or IV
non-small-cell lung cancer, provides motivation for the investigation of this TKI for use in breast
cancer as well.[37] Targeting Angiogenesis

Angiogenesis is another necessary aspect of cancer
development. As tumors grow, they are increasingly dependent on new blood vessel formation for
adequate oxygen and nutrients. A highly conserved, homodimeric member of the PDGF super-
family, vascular endothelial growth factor (VEGF) is the most potent and specific glycoprotein driving
tumor angiogenesis.[38] This heparin-binding glycoprotein also mediates vascular permeability and
induces endothelial migration. VEGF catalyzes signal transduction pathways very similar to those of
EGFR by binding one of three extracellular tyrosine kinase receptors: flt-1 (VEGFR-1), KDR (VEGFR-2),
or flt-4 (VEGFR-3). Intracellular signaling results in neovascular formation (Figure 3). In tumor cells, overexpression of VEGF results in abnormal, leaky vessels, with blind sacs and variations in flow as compared to normal vasculature.[39] Bevacizumab (Avastin) is a recombinant monoclonal antibody that binds directly to VEGF so that it is unable to bind any of its usual VEGF-Rs, thereby inhibiting angiogenesis. Preclinical studies with bevacizumab confirmed that VEGF does result in a reduction of tumor microvessel density, as well as a delay in tumor growth.[40] Other studies in murine models affirm that antiangiogenic agents will potentiate the antitumor effects of standard chemotherapy.[41] VEGF is highly expressed in the majority of cancers, including breast cancer.[42,43] In both node-positive and node-negative breast cancers, VEGF has been found to serve as a marker of larger tumors, p53 mutations, and poor tumor differentiation.[44] Phase I clinical trials demonstrate that bevacizumab is well tolerated, in comparison to many cytotoxic chemotherapies. Common side effects include hypertension, epistaxis, and proteinuria. More serious side effects such as thromboembolism and pulmonary hypertension are rare.[45] Because of its generally favorable tolerability, it has been readily combined with other chemotherapies in hopes of augmenting antitumor response, including a phase II trial in patients with previously treated metastatic breast cancer where using bevacizumab with vinorelbine resulted in a 31% objective response rate.[46] In a recent study in previously untreated women with inflammatory breast cancer treated with bevacizumab and vinorelbine, reduced VEGF levels were demonstrated, as well as a decrease in vascular permeability and endothelial cell proliferation as seen on dynamic contrast enhanced MRI.[47] A large prospective randomized phase III trial recently compared capecitabine (Xeloda) alone or in combination with bevacizumab in anthracycline- and taxane-pretreated metastatic breast cancer. The combination increased the response rate (19.8% vs 9.1%, \(P = .001\), yet there was no improvement in median time to progression or survival.[48] In May 2004, the Eastern Cooperative Oncology Group completed accrual for a phase III trial (study E2100) examining the addition of bevacizumab to weekly paclitaxel, as compared to paclitaxel alone for women with metastatic breast cancer. It is hoped that the results will demonstrate that this standard breast cancer therapy can be further augmented with the addition of bevacizumab. Recent evidence suggests that HER2 and VEGF signaling pathways also rely on "cross-talk" phosphorylation in human breast cancers in much the same way as the EGFR pathway has been linked to ER steroid hormone molecular signaling.[49] Gefitinib has been shown to inhibit production of tumor necrosis factor-alpha, bFGF, and VEGF in several human epithelial cancer cell lines.[50] Therefore, inhibition of both pathways is hypothesized to result in synergistic antitumor effects. A recently completed phase II trial at MSKCC and University of California-San Francisco examined the use of bevacizumab and erlotinib for their combined effects in metastatic breast cancer. **Targeting Cyclooxygenase-2**

Cyclooxygenase-2 (COX-2) is an inducible enzyme responsible for the rate-limiting conversion of arachidonic acid to prostaglandins in a variety of cellular perturbations such as inflammation and tissue damage.[51] Deregulation of COX-2 and downstream prostaglandins have also been demonstrated in tumorigenesis, and elevated COX-2 has been measured in a variety of epithelial carcinomas including colorectal,[52] lung,[53] esophageal,[54] and breast tumors.[55] Elevated levels of COX-2 mRNA, COX-2 protein, and PGE2 in colorectal carcinoma cells was linked to the activation of the HER2/HER3 pathway in colorectal carcinoma cells-an EGFR family pathway discussed above for its association with breast cancer pathogenesis.[56] Once again, "cross-talk" between the HER2 and COX-2 pathways appears to be associated with the development of breast cancer tumors expressing the HER2-neu oncogene. COX-2-selective inhibitors, such as celecoxib (Celebrex), have been shown to delay incidence of mammary tumors in transgenic mice overexpressing HER-2.[57] The mechanism of action by which COX-2 inhibitors slow tumor progression is still not well understood, but recently Chang et al proposed that by blocking PGE2, a potent inducer of angiogenesis and angiogenic regulatory proteins such as VEGF, COX-2 inhibitors cause apoptosis of the tumor microvasculature as well as tumor mass reduction. Chang et al suggested that, along with VEGF, COX-2 may therefore be another crucial component of the "angiogenic switch," necessary for tumor vessel formation.[56] Preclinical studies using a transgenic mouse model demonstrate a decrease in primary mammary tumor burden due to decreased tumor proliferation, as well as an increase in the induction of apoptosis when treated with COX-2 inhibitors.[57] Oral administration of celecoxib also demonstrated decreased expression of proangiogenic VEGF.
Based on these in vivo studies, an exploratory open phase I clinical trial using celecoxib (either 200 or 400 mg twice daily, orally) in breast cancer patients prior to surgery are currently under way (MSKCC 03-027). COX-2 expression, downstream signaling, as well as aromatase activity are all being examined (Figure 4). In another preliminary phase II trial at Ohio State University, celecoxib at two different doses is being studied as remission maintenance therapy after four to six cycles of chemotherapy (Cancer and Leukemia Group B 40105).

**Targeting a Favorable Genotype-DNA Profiling**

Given the variety of mechanisms targeted thus far in "rational" drug design, it is doubtful that any one targeted therapy will ever be sufficient to treat a disease as heterogenous as breast cancer. In order to best utilize targeted therapies, it will be important to expand upon the lessons learned with gefitinib by Lynch et al: while a particular therapy may only achieve marginal results when used in a random group of patients, in a more specific subset, the same therapy may be infinitely more potent. Genetic profiling methods such as cDNA microarray and RT-PCR array technology allow a more "personalized" approach to clinical decision-making not possible in the trial and error methods of standard chemotherapy regimens. DNA and reverse transcription polymerase chain reaction (RT-PCR) microarray allow a quantification of gene expression patterns across a large number of tissue samples. The relative patterns of overexpression, downregulation, or deletion can be computationally compared and grouped by profile similarities in order to examine differences between normal and malignant tissues. Microarray data can therefore also be queried to correlate specific expression patterns with survival or tumor progression, or poor prognosis.[58]

Using 70 genes associated with an increased risk of early metastasis in young patients with lymph node- negative breast cancer, van 't Veer and colleagues developed a geneexpression microarray to predict the likelihood of distant breast cancer metastasis.[59] When validated using a population of 295 patients, the 10-year survival rate was 55% for patients whose tumors had a "poor prognosis" gene expression pattern as compared to 85% for those with a "good prognosis" expression signature.[60] When validated in a population of 295 patients, this expression array was found to be a more powerful predictor of disease outcome in young patients with breast cancer than standard clinical and histological criteria alone. In the Microarray for Node-Negative Disease May Avoid Chemotherapy Trial (MINDACT), a large prospective clinical trial involving 21 countries in Latin America and Europe, 40 researchers are again using this genetic expression profile to compare its predictive powers with conventional histopathologic and clinical recurrence criteria.[61] Approximately 5,000 patients will participate in this trial. Because as many as 70% of these women with node-negative disease are cured by locoregional treatment alone,[62], yet > 95% women with node-negative breast cancer are still considered eligible for systemic chemotherapy, gene expression profiles have potential to spare women with a "good-prognosis" profile the toxic burdens of unnecessary chemotherapy. Paik et al used an RT-PCR array to identify genes with roles in cellular proliferation, invasion and hormone metabolism. From these initial gene candidates, 21 were selected (16 cancer- related, 5 reference genes) for the
development of a biomarker called the Oncotype DX gene assay to predict the chance of reoccurrence of breast cancers after hormonal adjuvant therapy[63] (Figure 5). This gene assay has been validated most recently[64] in a group of 675 patients with lymph node-negative, estrogen receptor-positive breast cancer from the National Surgical Adjuvant Breast and Bowel Project B-14 trial, which enrolled patients to randomly receive tamoxifen vs placebo as part of the study. Based on the relative levels of expression of the 16 selected cancer-related genes, patients were divided into groups of low, medium, or high risk of recurrence. The rate of 10-year distant recurrence in those patients with a low-risk recurrence score was 6.8%, while 14.3% of patients with a medium-risk recurrence score and 30.5% of patients with a high-risk score were found to develop recurrent disease. Thus, the recurrence score provided a significant predictive tool for determining recurrence, independent of the age and tumor size of the women who participated, and was also predictive of overall survival. Predictive tools such as the recurrence score have huge implications- not only to decide whether adjuvant therapy is appropriate, but also which therapy to use. Investigations are also under way to utilize expression profiles to predict tumor response to conventional therapy.

Pusztai et al used DNA microarray technology to develop a gene expression profile which demonstrated a 75% greater chance of achieving a complete pathologic response to neoadjuvant paclitaxel followed by T-FAC (fluorouracil [5-FU], doxorubicin [Adriamycin], cyclophosphamide) chemotherapy in patients with early-stage breast cancers.[65] Subsequently, Gianni et al used the same expression array to predict response to neoadjuvant chemotherapy with doxorubicin and paclitaxel in patients with locally advanced breast cancer.[66] A pathologic complete response in these patients was associated with higher expression of proliferation- and immune- related genes and with lower expression of estrogen-related genes.

Conclusions: The emergence of novel targeted therapies in breast cancer treatment has provided advances in treatment efficacy with minimal increase in toxicities, while at the same time yielding a better understanding of the responsible signal transduction mechanisms and the ways in which different pathways are connected. The optimal implementation of these drugs will likely require inhibition of multiple critical pathways, rather than any one "magic bullet" monotherapy. Just as important will be the use of expression profiles to predict patient prognosis and response of a particular genotype to a selected targeted therapy. In this manner, treating metastatic breast cancer will involve a personalized strategy for every patient: to favorably alter its natural history and possibly transform it to a chronic lifelong illness, or even a curable one.

Disclosures: Dr. Seidman has served on speakers’ bureaus for Abraxis Onclogy, Genentech, Bristol-Myers Squibb, Sanofi-Aventis, and Eli Lilly.

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