Traditional musician, Halong, Vietnam. Photograph courtesy of J. Bryan Murphy, MD, Clearwater, Florida.

Several promising agents in targeted treatment for breast cancer are discussed.

Molecularly Targeted Therapies for Breast Cancer

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Background: *The management of patients with localized and advanced breast cancer continues to evolve. Chemotherapy, endocrine therapy, and trastuzumab are effective therapies but leave considerable room for improvement. As the cellular aberrations inherent to cancer cells in general and breast cancer cells specifically are better understood, therapies to target specific cellular pathways continue to be developed with the goal of expanding available effective therapy through better patient selection.*

Methods: *We conducted a computerized search of the medical literature as well as a manual search of selected meeting abstracts.*

Results: *Several targeted therapies are in phase III clinical trials testing their promise in the treatment of breast cancer. Many other agents are completing phase I and II testing. An overview of the most promising agents in clinical development is discussed herein.*

Conclusions: *Targeted therapy for breast cancer is a reality at this time, and several new agents hold promise for expanding and refining the pool of patients likely to further benefit from this approach in the near future.*

Introduction

Despite advances in early detection of breast cancer, adjuvant therapy of localized disease, and palliative therapy of metastatic disease, breast cancer remains a significant public health problem, with an estimated 212,930 new cases and 40,840 deaths in the United States alone in 2005.[1] Cytotoxic chemotherapy remains an important part of optimal therapy for patients in all stages of disease, but it is limited by toxicity, nonspecificity, and inevitable development of resistance. Cytotoxic therapy has not been considered a "targeted" therapy since many of its specific targets have not been identified; it is evident that to be effective against cancer, it has to target cellular pathways involved in growth regulation. The term *targeted therapy*

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Abbreviations used in this paper: ER = estrogen receptor, PgR = progesterone receptor, HER = human epidermal growth factor receptor, AI = aromatase inhibitor, TK = tyrosine kinase, PI3K = phosphatidylinositol 3-kinase, IHC = immunohistochemistry, FISH = fluorescent in situ hybridization.

ideally connotes the ability to identify a known therapeutic target that is important in the biology of the cancer cell and use a specific agent that can treat the disease by modifying the expression or activity of the target in the growth and progression of the cancer. With this approach, only patients with a likelihood of benefit are treated, so the therapeutic index will hopefully be improved.

The story of selective and targeted therapy for breast cancer is an old one that involves the use of oophorectomy, hypophysectomy, and adrenalectomy as palliative and adjuvant endocrine therapy throughout the past century. With the discovery of the estrogen receptor (ER) and with our evolving understanding of the biology of this receptor pathway, the development of targeted agents to modulate the activity of this pathway (selective estrogen receptor modulators [SERMS]), to inhibit the production of the ligand (aromatase inhibitors), and to downregulate the receptor expression and activity (fulvestrant) has markedly improved outcomes of localized and advanced breast cancer expressing the ER.[2] Only patients who express the estrogen and/or progesterone receptor (PgR) benefit from endocrine therapy, although identification of other predictors of benefit to endocrine therapies is still needed as only approximately 30% of patients receiving first-line endocrine therapy for metastatic disease will achieve partial or complete regression of disease with such therapies.

The humanized monoclonal antibody trastuzumab was developed as a therapy targeted against the human epidermal growth factor receptor 2 (HER2), which is overexpressed in roughly one fourth of patients with invasive breast cancer. Readily available markers of overexpression and/or gene amplification of HER2 in tumor tissue predict for the activity of this agent and exclude those who will not benefit from this therapy. Randomized trials have demonstrated a survival benefit associated with the introduction of this agent in addition to chemotherapy in women who have metastatic breast cancer that overexpresses the HER2 protein or have amplification of the HER2 gene.[3,4] It is hopeful that ongoing trials of this agent in the adjuvant setting will also demonstrate an improvement in survival with the addition of this agent to chemotherapy. Other than concerns of cardiac toxicity, the tolerability of this agent is excellent.

Although these examples of targeted therapy for breast cancer using antiestrogen or anti-HER2 therapies have been successful, there is more to be done. Many patients have tumors that do not express either steroid hormone receptors or HER2. Resistance to trastuzumab and endocrine therapy develops in essentially all patients with advanced disease. As the understanding of the biology of breast cancer evolves, several other important intracellular pathways have been discovered as targets for novel therapeutic agents, including other members of the HER family, proangiogenic pathways, proliferative pathways, pathways of cell-cycle regulation, apoptosis pathways, and many others. It is also becoming clearer that the complex interplay of these pathways will likely require multiple targets to be inhibited to optimize cytotoxicity and overcome resistance mechanisms. This review highlights agents that are furthest along in their clinical development as breast cancer therapeutics.

Targeted Endocrine Therapy of Breast Cancer

Tamoxifen

Tamoxifen has arguably been the most successful targeted therapy for malignancy for decades. In the adjuvant setting, 5 years of tamoxifen use significantly reduces the risk of recurrent breast cancer and improves survival for both premenopausal and postmenopausal patients with ER+ resected breast cancer. Tamoxifen also reduces the incidence of new primary breast cancer by almost 50%.[5] In the metastatic setting, objective responses range from 25% to 50%, and additional patients benefit with prolonged stability of disease.[6,7]

Aromatase Inhibitors

Randomized trials of postmenopausal patients with metastatic disease have shown the third-generation nonsteroidal aromatase inhibitors (AIs) anastrozole and letrozole, as well as the steroidal AI exemestane, to be superior to megestrol acetate in patients with disease progression after tamoxifen, as well as better tolerated. In the first-line setting, anastrozole, letrozole, and exemestane have demonstrated superior time to progression compared with tamoxifen.[2]

Adjuvant and neoadjuvant trials comparing AI therapy to tamoxifen have been reported and might shed light on using molecular targets to potentially select which patients may benefit most from AIs compared to tamoxifen. In a subgroup analysis of a phase III neoadjuvant trial comparing 4 months of letrozole to tamoxifen, patients who were HER1+ and/or HER2+ had an improved response rate with letrozole (88% vs 21%).[8] The IMPACT investigators performed a neoadjuvant trial of tamoxifen vs anastrozole vs the combination for 3 months. Overall, there was no difference in the primary endpoint of objective response. Data were presented for a small subgroup of patients with HER2+ breast cancer. There were 7 responses out of 12 patients treated with anastrozole compared with 2 responses out of 9 patients treated with tamoxifen.[9]

In the ATAC trial involving 9,366 women with resected breast cancer randomized to anastrozole, tamoxifen, or the combination, 7,839 were known to be hormone receptor-positive. For these patients there is a statistically significant improvement in disease-free survival favoring anastrazole over tamoxifen (hazard ratio 0.83, P=.013).[10] A subgroup analysis of these patients was performed with the hypothesis that the effect of anastrozole would differ based on PgR status. In the 5,704 patients with both ER+

and PgR+, the hazard ratio was 0.82, though this was not statistically significant. In the 1,370 women who were ER+ but PgR–, the hazard ratio was 0.48 ($P<.001$), suggesting a striking improvement in outcome for these patients.[11] However, this reported role of PgR expression in ATAC was based on a retrospective analysis, and it has not yet been demonstrated in the other adjuvant AI trials (IES, ABCSG8/ARNO95, or BIG 1-98). Thus, the relationship between PgR expression and benefit to AIs in the adjuvant setting is unclear at this time.

The above discussion, as well as a growing body of retrospective and preclinical evidence, suggests that there may be a way of selecting the most active endocrine therapy based on tumor molecular characteristics and also suggests mechanisms for endocrine therapy resistance. These hypotheses continue to be the subject of ongoing prospective trials.

Targeting the HER Receptor Family

The HER family of transmembrane receptors consists of 4 closely related members, including HER1 (also known as the epidermal growth factor receptor [EGFR]), HER2, HER3, and HER4. In general, they consist of an extracellular ligand binding domain, a transmembrane domain, and an intracellular tyrosine kinase (TK) domain. With binding of ligand, HER family homodimers or heterodimers are formed with resulting activation of the intracellular TK domain by phosphorylation as well as an increased affinity for ligand binding. Activation of HER TKs leads to protean changes in many intracellular pathways related to proliferation, angiogenesis, apoptosis, and other cellular effects. Several ligands have been found that bind HER1, HER3, and HER4, including transforming growth factor alpha (TGFα), epidermal growth factor (EGF), and heregulins. No known natural ligand exists for HER2, but evidence suggests it is the preferred dimerization partner for activation of the other HER receptors. The intracellular component of HER3 lacks TK activity but contains multiple binding sites for phosphatidylinositol 3-kinase (PI3K) and is a potent activator in partner with HER1 and HER2 of this signal pathway associated with cell survival and proliferation.[12]

Preclinical and clinical evidence suggests that overexpression of HER2 is an early event in tumorigenesis, and it can be important for tumor growth and unchecked progression through the cell cycle. HER2 overexpression by gene amplification occurs in approximately 20% of breast cancers. HER2 overexpression is associated with aggressive biology and poor prognosis in resected and metastatic breast cancer. Overexpression of HER1 appears to be a later event in the evolution of the cancer cell phenotype. Overexpression of HER1 is found in about 40% of breast cancers and is associated with increased proliferation, increased angiogenesis, and decreased apoptosis. Overexpression of HER1 also portends a poor prognosis in breast cancer.[13] Overexpression of either HER1 or HER2 is associated with relative resistance to endocrine therapy.[12] Thus, this family of receptor TKs is a logical and important target for breast cancer therapy.

HER2 Inhibition

Trastuzumab is a recombinant, humanized monoclonal antibody targeted against the extracellular domain of HER2. The binding of trastuzumab is thought to exercise its therapeutic effects through multiple mechanisms. Preclinical evidence supports the induction of antibody-mediated cytotoxicity as one mechanism. The binding of trastuzumab to HER2 also inhibits signaling through other members of the HER family by inhibition of formation of the heterodimers important for the potentiation of HER signaling. This can result in decreased angiogenesis, increased apoptosis, and decreased proliferation.[14]

In properly selected patients, trastuzumab has single agent activity as well as potent synergy with many chemotherapy agents in metastatic breast cancer. It is now clear that 3+ expression by standardized immunohistochemistry (IHC) methods or gene amplification demonstrated by fluorescent in situ hybridization (FISH) is necessary for likely benefit from trastuzumab.[15] Early studies of this agent included patients who were graded 2+ or 3+ by IHC, and these studies likely underestimate the benefits of this agent.

Cobleigh et al[16] reported result of single-agent trastuzumab in 222 women with metastatic breast cancer previously treated with one or two regimens for metastatic disease. All patients received the standard dose of 4 mg/kg intravenously (IV) as an initial loading dose and 2 mg/kg IV weekly thereafter. An objective response rate of 15% was seen, with a median duration of response of 9.1 months in this refractory population. Tolerance was excellent, with the exception of mild infusion reactions with the first dose and cardiac dysfunction in 4.7% of patients.

The pivotal trial evaluating trastuzumab (T) in combination with chemotherapy randomized 469 women with previously untreated metastatic breast cancer to chemotherapy alone vs chemotherapy plus trastuzumab. Patients received standard doses of doxorubicin and cyclophosphamide (AC) unless they had received an anthracycline in the adjuvant setting. These patients received paclitaxel at a dose of 175 mg/m^2 every 3 weeks with or without trastuzumab. The addition of trastuzumab to chemotherapy resulted in an increased response rate (50% vs 32%, $P<.001$), a longer median duration of response (9.1 months vs 6.1 months, $P<.001$), and prolonged overall survival (median 25.1 months vs 20.3, $P=.046$). Toxicity was similar in the two groups, with the important exception of increased cardiac toxicity. With the combination of AC plus trastuzumab, 27% of patients had cardiac dysfunction compared with 8% of patients

who received AC alone. The combination of paclitaxel and trastuzumab resulted in cardiac dysfunction in 13% of patients, while only 1% of patients receiving paclitaxel alone had cardiac dysfunction. Class III or IV heart failure (based on the New York Heart Association classification) was reported in 16% of patients with AC plus trastuzumab, in 3% with AC, in 2% with paclitaxel plus trastuzumab, and in 1% with paclitaxel alone.[2] As a result of this trial, all patients with HER2 overexpressing metastatic breast cancer should receive trastuzumab in addition to their palliative chemotherapy, but anthracycline/trastuzumab combinations should be avoided outside of a clinical trial. Several other chemotherapeutic agents have been subsequently shown to have excellent preclinical and clinical synergy with trastuzumab.[17]

Vogel et al[15] evaluated single-agent trastuzumab in the first-line setting in 114 women with metastatic breast cancer. All patients had tumors scored as 2+ or 3+ by IHC. Two different dose levels were evaluated, but no difference in outcome was observed for the higher dose compared with the standard dose. The objective response rate for the whole population was 26%, with 38% having clinical benefit (response or stable disease for 6 months). Roughly half of patients with a clinical benefit or response were free of disease progression at 1 year. Median survival was 24.5 months, which rivals the chemotherapy plus trastuzumab result from the pivotal trial with a similar population. In an analysis of patients with 3+ expression of HER2 by IHC, the clinical benefit rate was 48%. Also, patients with gene amplification by FISH had an objective response rate of 34%. Cardiac toxicity was rare in this trial. This study supports the potential use of trastuzumab alone as initial therapy for patients with HER2 overexpressing metastatic breast cancer and suggests that survival may not be impaired by withholding chemotherapy initially, though cross-study comparisons such as this are fraught with potential bias.

Baselga and colleagues[18] reported on the efficacy and safety of every-3-week trastuzumab monotherapy in patients with HER2+ metastatic breast cancer who had not received prior chemotherapy for metastatic disease. HER2 positivity was defined as 3+ by IHC or positive by FISH. A total of 105 patients with a median number of metastatic sites of 2 were enrolled (22 were not evaluable as they did not have HER2+ tumor or measurable disease). In 83 patients with proven HER2+ measurable disease, the response rate to trastuzumab (8 mg/kg loading dose followed by 6 mg/kg dose every 3 weeks) was 24%. If patients who achieved disease stabilization for 6 months or longer are added, the clinical benefit rate was 36%. The median duration of response was 10.1 months, with a median time to response of 1.4 months. Safety analysis demonstrated that 16% of assessed patients had decreases of left ventricular ejection faction (LVEF) of 15% or more, and 13% had an absolute value below 50%. Only 1 patient had symptomatic cardiac event. This study concluded that the safety and efficacy of every-3-week trastuzumab are similar to those reported with weekly trastuzumab, but this new schedule can improve patient convenience, which is particularly relevant in patients receiving this treatment for prolonged periods of time.

Given the excellent activity and tolerability of trastuzumab in the metastatic setting, extensive evaluation of this agent in the adjuvant setting is underway (Table 1). Two large North American studies are nearing completion of accrual. The North Central Cancer Treatment Group (NCCTG) N9831 intergroup trial involves a control arm of AC every 3 weeks for 4 cycles followed by a dose-dense schedule of weekly paclitaxel for 12 weeks. The two experimental arms involve 1 year of weekly trastuzumab in addition to the chemotherapy, starting either with paclitaxel or at the completion of the paclitaxel schedule. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial includes a control arm of AC every 3 weeks for 4 cycles followed by paclitaxel every 3 weeks for 4 cycles, with the experimental arm receiving weekly trastuzumab for 1 year after chemotherapy. A cooperative effort between the leaders of these two trials is underway to combine the data of the trastuzumab-containing arms vs control arms in an effort to obtain an earlier answer as to the potential benefit of trastuzumab in the adjuvant setting. Two large international trials are also evaluating the role of adjuvant trastuzumab. The Breast Cancer International Research Group (BCIRG) 006 trial includes a control arm of AC for 4 cycles followed by docetaxel for 4 cycles. One experimental arm adds 1 year of trastuzumab, while the other evaluates the novel adjuvant chemotherapy combination of docetaxel and carboplatin for 6 cycles with concurrent and sequential trastuzumab for 1 year. The Herceptin Adjuvant (HERA) trial allows any acceptable chemotherapy to be given, followed by a randomization to trastuzumab for 1 or 2 years or observation.

Table 1. — Trials of Adjuvant Trastuzumab

Trial	Schema	
NCCTG N9831	AC × 4 q 3 w →	Arm 1: Paclitaxel q w × 12 Arm 2: Paclitaxel q w × 12 → trastuzumab q w × 52 Arm 3: Paclitaxel/trastuzumab q w × 12 → trastuzumab q w × 40
NSABP B-31	AC × 4 q 3 w →	Arm 1: Paclitaxel × 4 q 3 w Arm 2: Paclitaxel × 4 q 3 w → trastuzumab q w × 52
BCIRG 006		Arm 1: AC × 4 → docetaxel × 4 Arm 2: AC × 4 → docetaxel × 4 → trastuzumab q 3 w × 1 yr Arm 3: Docetaxel/carboplatin/ trastuzumab q 3 w → trastuzumab q 3 w to complete 1 yr
HERA	Any acceptable → chemotherapy	Arm 1: observation Arm 2: trastuzumab × 1 yr Arm 3: trastuzumab × 2 yrs

AC = doxorubicin and cyclophosphamide

HER1 (EGFR) Inhibition

Two main classes of agents have been developed that specifically target the EGFR. Gefitinib and erlotinib are small molecule inhibitors of the EGFR TK, and cetuximab is among a group of monoclonal antibodies that target EGFR. Albain and colleagues[19] reported a 4.8% clinical benefit rate (complete response, partial response, or stable disease longer than 6 months) for gefitinib in a highly refractory population. Robertson et al[20] reported a clinical benefit at 6 months in 5 (26%) of 19 patients treated with gefitinib. Baselga et al[21] treated 31 patients with gefitinib and reported a 13% clinical benefit rate. A phase II trial of erlotinib similarly demonstrated a low clinical benefit rate (less than 5%) in refractory patients.[22] These studies demonstrate some single-agent activity of the small molecule TK inhibitors in refractory metastatic breast cancer, but they are somewhat disappointing. A significant problem with anti-EGFR therapy is that a good predictive marker for benefit is not readily available to ensure that patients treated are those in whom the target is an important part of the tumor biology. No studies have been reported as yet with anti-EGFR monoclonal antibodies, and it should be noted that there has been little activity of the small molecule TK inhibitors in metastatic colon cancer, yet reproducible activity has been observed with monoclonal antibodies such as cetuximab, which may have multiple mechanisms of action beyond inhibition of the EGFR TK.[23]

There are good preclinical and some clinical data regarding the synergy of anti-EGFR therapy and chemotherapy,[24] and this strategy is being pursued in breast cancer. The NCCTG has completed a trial of gemcitabine and erlotinib and will soon open a trial of irinotecan and cetuximab in the setting of refractory metastatic breast cancer. Other research strategies ongoing with these agents include combinations with other targeted therapies, including endocrine therapy.

Inhibitors of Multiple HER Receptors

Inhibiting multiple HER receptors has the potential to improve therapy by intensifying and broadening the inhibition of multiple and redundant cellular pathways. Several compounds have been developed that have broad anti-HER activity (Table 2).

Lapatinib (GW572016)

Lapatinib is a small molecule inhibitor of the TK domain of both HER1 and HER2. Preclinical studies have demonstrated inhibition of growth and induction of apoptosis in breast cancer cells lines driven by EGFR or HER2 expression through decreased phosphorylation of the TK domains of both receptors. Lapatinib has a significantly longer half-life than gefitinib or erlotinib.[25,26] Burris et al[27] reported a phase 1b trial of lapatinib in EGFR and/or HER2 expressing cancers. Sixty-six patients were treated, including 30 patients with metastatic breast cancer. Four of the patients with metastatic breast cancer (13%) had an objective response. All four had progressed through prior trastuzumab. Ten patients (33%) were believed to have stable disease at a median duration of therapy of 5 months. All 10 had EGFR expression by IHC, and 8 of these 10 overexpressed HER2.[27,28]

Preliminary results of a phase II trial of lapatinib in trastuzumab-refractory HER2+ metastatic breast cancer have been reported. Of 41 patients evaluable at the time of this report, the objective response rate was assessed as 10%, though only a 5% response rate was confirmed by an independent review. The same independent review confirmed disease stabilization at 16 weeks in 25% of patients. Grade 3 toxicity was modest and consisted of diarrhea, rash, and fatigue in 10% or less of patients.[29,30]

Based on the modest single-agent activity of this drug in patients with multiple prior trastuzumab-containing therapies, several phase III trials are planned or ongoing to study this drug in addition to capecitabine, the taxanes, and letrozole. These are mainly in the metastatic setting, but a new adjuvant (and neoadjuvant) trial is under development by the NCCTG and the North American Breast Intergroup incorporating this agent for HER2+ patients.

Pertuzumab (2C4)

Pertuzumab is a well tolerated monoclonal antibody designed to target HER2 at an epitope distinct from trastuzumab. It blocks formation of HER2 heterodimers with other members of the HER family and thus reduces signaling through the multiple pathways associated with HER activation.[31] A phase II study of this agent in metastatic breast cancer has completed accrual but has yet to be reported.

Canertinib (CI-1033)

Canertinib is an orally available pan-HER TK inhibitor. In addition, its binding to the TK sites is irreversible, which

Table 2. — Agents Targeting HER Signaling

Drug	Target				Tyrosine Kinase Inhibitor	Monoclonal Antibody
	HER1	HER2	HER3	HER4		
Trastuzumab	–	+	–	–	–	+
Gefitinib, erlotinib	+	–	–	–	+	–
Cetuximab	+	–	–	–	–	+
Lapatinib	+	+	–	–	+	–
Pertuzumab*	+	+	+	+	–	+
CI-1033	+	+	+	+	+	–

* Binds to HER2 but inhibits HER2 dimerization with HER1, HER3, HER4.

may be an advantage compared to other TK inhibitors that bind reversibly. Canertinib has been well tolerated in a variety of phase I schedules with some disease stabilization in patients with refractory metastatic breast cancer.[32] A phase II trial of this agent in metastatic breast cancer has completed accrual.

Combination of HER Signaling Inhibitors

A phase I/II study performed by the Eastern Cooperative Oncology Group (ECOG) has been completed on the combination of gefitinib and trastuzumab. In the phase II portion of the trial using standard doses of trastuzumab and 250 mg/day of gefitinib, median time to progression was 2.9 months in a chemotherapy-naive subset and 2.2 months in those with prior therapy. These results appear to be inferior to those with trastuzumab as a single agent. The authors suggest that increased phosphorylation of HER3 with resulting signaling through the PI3K pathway occurs in the setting of trastuzumab and gefitinib as a combination, but not when used alone in preclinical models, which may be one reason for this disappointing result.[33]

Burris and coworkers[34] presented results of a phase I open label trial of oral lapatinib in combination with trastuzumab in patients with HER2+ metastatic breast cancer (2–3+ by IHC or FISH+). Escalating doses of lapatinib (750 to 1,500 mg/day) were administered with weekly trastuzumab. The optimal tolerated regimen was defined as lapatinib 1,000 mg/day combined with standard trastuzumab (4 mg/kg loading followed by 2 mg/kg weekly). A total of 48 heavily pretreated patients were included, with 45 having received prior trastuzumab therapy. Six (22.5%) of the 26 evaluable patients responded (1 complete response and 5 partial responses). No patient on the study had a symptomatic or asymptomatic decline in left ventricular ejection faction (LVEF).

Arpino et al[35] presented preclinical data at the same San Antonio Breast Cancer Symposium as the two previous studies from a mouse model of tamoxifen-stimulated HER2+ breast tumor xenografts. Delays in tumor growth and some responses were demonstrated by adding either gefitinib, trastuzumab, or pertuzumab to the model. Improved outcome was seen with HER-signaling inhibitor doublets in combination with tamoxifen. The most dramatic effect, however, was seen when all agents were combined in a "complete blockade" of HER signaling, with 90% complete response and no tumors progressing after 129 days of follow-up. Further refinement of the optimal approach to combine these agents clinically will be an important ongoing research strategy.

Antiangiogenic Agents

The important role that tumor angiogenesis plays in the growth of tumor metastases larger than 2 to 3 mm has been established.[36] In breast cancer, angiogenesis is important in the growth and progression of metastatic lesions. It is upregulated by activated HER1 and HER2 signaling. Vascular endothelial growth factor (VEGF) is a potent inducer of endothelial cell migration, invasion, vascular permeability, and vessel formation. VEGF is also a survival factor for endothelial cells.[37]

Bevacizumab is a recombinant humanized anti-VEGF antibody. The antibody blocks binding of VEGF to its receptors on endothelial cells as measured in preclinical models. A randomized phase III trial in the first-line therapy of metastatic colorectal cancer demonstrated improvement in response, duration of response, and overall survival with the addition of bevacizumab to chemotherapy vs chemotherapy alone.[38] It should be noted that recently, an increase in arterial thrombotic events has been reported in patients treated on trials across all diseases in patients who receive bevacizumab as compared to those who do not (NCI communication, unpublished data, 2004).

A phase I/II study of single-agent bevacizumab enrolled 75 patients with refractory metastatic breast cancer.[39] In all patients, an objective response rate of 6.7% was confirmed, and 17% of patients were still being treated with response or stable disease at day 154 of assessment. Hypertension, nephrotic syndrome, headache, and thromboembolic disease were the main serious adverse events reported in this trial, which were similar in other trials of this agent.

Subsequently, a randomized phase III trial of capecitabine vs capecitabine plus bevacizumab was reported with disappointing results.[40] In this population of extensively pretreated patients with metastatic breast cancer, the primary endpoint of progression-free survival was not improved with the addition of bevacizumab, nor was survival. An increase in objective response rate was seen from 9% for capecitabine alone to 20% for the combination.

ECOG 2100, A randomized phase III trial in patients with previously untreated metastatic breast cancer has completed accrual. This study randomized patients to weekly paclitaxel with or without bevacizumab. It is hoped that additive benefit will be demonstrated in this more favorable patient population.

Other antiangiogenic agents are in development including inhibitors of the VEGF-receptors TK alone or in combination with other receptor TKs, such as SU11248, which inhibits the TK activity of VEGF-R, platelet-derived growth factor receptor (PDGF-R), c-kit, and flt3.

Ras/Raf/MEK/ERK Pathway

The Ras family of guanine nucleotide binding proteins is integral in normal cell proliferation. When activated, they localize to the inner surface of the plasma membrane and activate many cytoplasmic and nuclear pathways involved in proliferation, cell survival, and cytoskeletal alterations. Oncogenic Ras mutations are rare in breast cancer, but

overexpression of Ras has been demonstrated at the protein and RNA level. Ras proteins are stimulated by several growth factor receptors such as HER1, HER2, insulin-like growth factor 1 (IGF-1), and ERα, all of which can be activated in human breast cancer. Activation of Ras activates the Raf-1 kinase, which in turn activates the MAP kinases MEK1 and MEK2. MEK activation turns on the ERK family of kinases, which translocate into the nucleus to broadly activate genes involved in proliferation. In addition, Ras can activate the PI3K pathway involved in cell survival and proliferation and the Rac and Rho proteins that are associated with the regulation of the cytoskeleton, perhaps associated with increased invasiveness of a tumor cell.[41]

The enzyme farnesyltransferase is involved in the initial posttranslational modification of Ras that allows it to become associated with the plasma membrane and become active in signal transduction. Inhibition of this enzyme is thus a logical therapeutic target for malignancies associated with activated ras.[42] There are also several other proteins involved in the regulation of the cell that also require farnesylation, and thus farnesyltransferase inhibitors (FTIs) may have broad antitumor effects beyond those mediated through the Ras pathways.

Farnesyltransferase Inhibitors

Several FTIs are in preclinical and clinical development and have broad preclinical activity against breast cancer cell lines and xenografts. There appears to be synergistic benefit with cytotoxic agents as well. R115777 (Zarnestra) is the agent furthest along in development. A phase II trial in patients with metastatic breast cancer, most having received prior endocrine and/or chemotherapy for advanced disease, has been reported.[43] A continuous and intermittent dosing schedule was investigated in 41 and 35 patients, respectively. The objective response rates in the 2 groups were 10% and 14%, with an additional 15% and 9% with stable disease for 6 months or more. The main serious toxicity observed was myelosuppression and neuropathy, both of which were less in the intermittent dosing group. Several phase I studies of this and other FTIs have been performed in conjunction with cytotoxic chemotherapy demonstrating the safety of these combinations,[37] and phase II trials in breast cancer are proceeding. Also a phase II trial in combination with an AI is also ongoing.

Raf Inhibitors

Compounds that inhibit Raf, a downstream effector of Ras, are also in development. ISIS 5132 is an antisense oligonucleotide targeting c-Raf and has undergone phase I evaluation with modest toxicity, and phase II trials are underway in a variety of tumor types. Bay 43-9006 is a small molecule inhibitor of B-Raf that has shown clear antitumor effects in a wide variety of tumor types in phase I trials.[37] It has also become more clear that several other receptor TKs are inhibited by this molecule, including VEGF-R2 and -R3, PDGF-R, c-*kit*, and *flt-3*, along with p38α, a member of the MAP kinase family (Bayer investigator's brochure 2003, unpublished data). We at NCCTG are currently conducting a phase II trial of this compound in patients with previously treated metastatic breast cancer.

MEK Inhibitors

CI-1040 is an oral, selective small-molecule inhibitor of MEK 1-2. Preclinical activity has been demonstrated in breast cancer cell lines, and mild gastrointestinal, skin, and constitutional side effects were observed in phase I.[44] A phase II study in 4 tumor types included a cohort of 14 patients with metastatic breast cancer treated with two prior chemotherapy regimens for metastatic disease. In this refractory population, no responses were seen though 1 patient had stable disease.[45]

Mammalian Target of Rapamycin and the PI3K/Akt Pathway

The PI3K pathway is involved in regulating multiple cellular functions important for cell survival and proliferation. Signaling through Akt regulates the serine-threonine kinase mammalian target of rapamycin (mTOR). mTOR is involved in transcriptional and translational regulation proteins important to regulation of the cell cycle. In breast cancer, the PI3K/Akt/mTOR pathway can be activated by the ER, the IGF-1 receptor, and the HER family, especially HER3. Oncogenic ras is another stimulator of this pathway.[46] Also, in a study of 70 primary breast cancer specimens, 40% had an activating mutation in the PI3KCA gene.[47] Rapamycin has immunosuppressive, fungicidal, and antitumor activity through inhibition of mTOR. Rapamycin targeting of mTOR results in cell-cycle arrest in G_1. Analogs of rapamycin have been developed to overcome problems with poor solubility and chemical stability of this agent, including CCI-779, RAD001, and AP23573. CCI-779 is furthest along in development.[46]

CCI-779

CCI-779 has been found to have activity in breast cancer cell lines, especially those that are estrogen dependent or overexpress HER2 or those with deletions of the tumor suppresser gene PTEN, which results in increased signaling through mTOR. Synergy with ER antagonists has also been demonstrated.[46]

A multicenter phase II study of two dose levels (75 mg and 250 mg) of CCI-779 was conducted in Europe in patients with anthracycline and/or taxane-refractory metastatic breast cancer. Among 98 evaluable patients, 10% had a partial response, and an additional 27% had stable disease for 8 weeks. Activity was observed at both doses. Tolerability was better at the lower dose, but both dose levels were generally well tolerated. Among the 59 patients evaluated for HER2 expression, no responses were seen in the

33 patients who were HER2–. In the 26 patients with overexpression of HER2, the response rate was 15%.[48] This may suggest further development in HER2+ breast cancer, perhaps combined with an anti-HER2 agent.

Based on the clinical and preclinical data, inhibitors of mTOR are promising candidates for breast cancer therapy and should be studied in combination with HER inhibitors as well as endocrine therapy, based on cross-talk between the ER pathway and the PI3K/Akt/mTOR pathway. Preliminary data from a randomized phase II trial of letrozole with or without CCI-779 have been reported.[49] Patients were randomized to letrozole alone, letrozole plus continuous CCI-779, or letrozole plus intermittent CCI-779. Stomatitis and diarrhea in the combination arms led to dose reductions of the CCI-779 for subsequent patients. Clinical activity has been observed in all arms, and the trial will lead into phase III study.[49] Trials in combination with chemotherapy are also contemplated.

The Ubiquitin-Proteasome Pathway

The ubiquitin-proteasome pathway is involved in the ordered degradation of key cell-cycle regulatory proteins and thus helps to govern transcription, the cell cycle, apoptosis, and angiogenesis. Multiple ubiquitin molecules bind to the protein substrates that are subsequently degraded by the multicatalytic proteasome complex. Inhibition of the proteasome complex has many effects on the cell. Among these are stabilization of p21, p27, and p53, which help to regulate the cell cycle and increase apoptosis as well as I-kappa B (IκB), which inhibits activation of the nuclear transcription factor kappa B (NF-κB). Activation of NF-κB promotes tumor cell survival and resistance to cytotoxic chemotherapy. Proteasome inhibition has been shown to increase chemosensitivity in preclinical models.[50]

PS-341 is a potent and specific inhibitor of the proteasome. Preclinical activity has been observed in breast cancer cell lines. A phase II study of this agent as monotherapy in patients with refractory breast cancer has been reported. There was no significant activity seen in the first 12 patients enrolled, and the study was closed.[51] Further exploration of this agent in combination with chemotherapy is ongoing.

Future Directions: Inhibiting Multiple Pathways and Refining the Targets

The ongoing significant improvements in our understanding of the altered molecular events in cancer cells have led to an explosion of new targets and agents for clinical testing. The examples of the targeting of the ER and HER2 pathways give hope for defining populations most likely to benefit from a targeted agent that may work as a single agent and may also increase the effectiveness of existing chemotherapeutic agents. The redundancy of cellular pathways and the multiple concurrent aberrations in cancer cells make it likely that multiple targets will need to be addressed for maximal clinical effect. In addition, many of the agents discussed in this review have poorly defined, validated, and identifiable targets in individual patients as of yet. This hampers the optimal development of these agents, as patient selection will be critical to detecting activity of an agent that works only in tumors with specific biologic characteristics. Clinical trial design will need to try to identify biologic assays that can measure the effects of these agents on the targeted pathways as a result of therapy and further refine their usage. Trials of new agents in the neoadjuvant setting may be one way to validate therapeutic targets with the opportunity of obtaining serial biopsies for putative biomarkers of drug activity and correlating with response. However, a caveat remains that improved response and/or biologic end points in the neoadjuvant setting have not been conclusively proven to predict survival benefit in the adjuvant setting.

It may be that single-agent testing of these drugs in refractory populations is not the best way to identify clinical benefit, and rational testing of combinations with chemotherapy, endocrine therapy, and radiation therapy are needed to exploit the possible synergies of these approaches. In addition, the known "cross-talk" between several of these pathways can suggest rational combinations for testing to maximize the inhibition of aberrant cellular signaling. Ideally this can be facilitated by good preclinical models of agent synergy. Clinical research is moving forward in an exciting direction of targeted therapy combinations for patients with metastatic breast cancer, and we can be hopeful that the promise of ER and HER2 targeted therapy will be expanded to patients with different biologic subsets of disease with some of the agents described in this review.

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