Emerging Targeted Therapies for Breast Cancer

Ricardo H. Alvarez, Vicente Valero, and Gabriel N. Hortobagyi

See accompanying articles on page 3248 and 3256

ABSTRACT

Increased understanding of the molecular events involved in cancer development has led to the identification of a large number of novel targets and, in parallel, to the development of multiple approaches to anticancer therapy. Targeted therapy focuses on specific molecules in the malignant cell signal transduction machinery, including crucial molecules involved in cell invasion, metastasis, apoptosis, cell-cycle control, and tumor-related angiogenesis. In breast cancer, two new targeted agents have recently been approved: lapatinib, directed against the human epidermal growth factor receptor 2 (HER2); and bevacizumab, directed against vascular endothelial growth factor (VEGF). Multiple other targeted agents are under evaluation in clinical trials, including inhibitors of the epidermal growth factor receptor (EGFR), dual EGFR and HER2 inhibitors, other VEGF or VEGF-receptor inhibitors, and agents that alter crucial signaling pathways, such as RAS/MEK/ERK; phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin; insulin-like growth factor/insulin-like growth factor receptor; poly (ADP-ribose) polymerase 1; and others. In this review, we present the most promising studies of these new targeted therapies and novel combinations of targeted therapies with traditional cytotoxic agents.

INTRODUCTION

Although numerous systemic agents are available to treat metastatic breast cancer (MBC), most tumors eventually become unresponsive to systemic therapy. In recent years, several targeted agents have become available that have improved the outcomes of patients with solid tumors. One of these agents, trastuzumab (Herceptin; Genentech, South San Francisco, CA), a monoclonal antibody against the human epidermal growth factor receptor 2 (HER2), has proven effective in the treatment of women with HER2-positive breast cancer.1-4 Other targeted agents are also showing promise in breast cancer treatment.

Two—lapatinib (Tykerb; GlaxoSmithKline, Research Triangle Park, NC), a selective, reversible dual inhibitor of the epidermal growth factor receptor (EGFR; HER1) and HER2, and bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF)—have recently been approved by the US Food and Drug Administration for patients with certain types of breast cancer.5,6 However, most targeted agents that have shown promise against breast cancer are still in preclinical or early clinical testing. Here, we review the most current information regarding the emerging targeted therapies for breast cancer. We excluded trastuzumab from this review, because its role in breast cancer is well established.
for breast cancer have been completed (Appendix Table A1, online only). Single-agent gefitinib showed minimal clinical benefit (CB). Although the studies of combination therapy were not randomized, gefitinib did not significantly improve overall response rate and time to treatment failure on a chemotherapy regimen.

More recently, an exploratory analysis of two randomized, phase II trials comparing anastrozole or tamoxifen plus gefitinib versus anastrozole or tamoxifen plus placebo was published.16 In both trials, endocrine-therapy–naïve patients had longer progression-free survival (PFS) with hormonal therapy plus gefitinib.

PR.19 The regimen was generally well tolerated; manageable skin and gastrointestinal problems were the most common treatment-related adverse effects. Several other preliminary studies of erlotinib combined with docetaxel,20 vinorelbine plus capecitabine,21 and bevacizumab22 have been reported.

On the basis of data from a preclinical mouse xenograft model, patients with stages I to IIIA invasive breast cancer were treated with erlotinib 150 mg/d orally for 6 to 14 days until the day before surgery.23 Ki67 expression was reduced in estrogen–receptor–positive tumors but not in tumors that overexpressed HER2 or were TRN.

Trastuzumab-DM1 is the first antibody–drug conjugate that is based on trastuzumab. Trastuzumab-DM1 consists of trastuzumab linked to an antimicrotubule drug, maytansine (also known as DM1). Trastuzumab-DM1 showed activity in a xenograft model of HER2-positive, trastuzumab-resistant tumors.24 A phase I study of trastuzumab-DM1 in heavily pretreated patients with HER2-overexpressing MBC showed clinical activity, with thrombocytopenia as the DLT, at a dosage of 4.8 mg/kg every 3 weeks. The recommended dosage for phase II studies was 3.6 mg/kg every 3 weeks.25 In a recent preliminary report of a phase II study of trastuzumab-DM1 in 112 patients with HER2-overexpressing MBC in whom treatment with trastuzumab, lapatinib, or both had failed to show promising activity, the independent review panel confirmed an overall response rate of 25% (28 patients) and a CR rate of 34% (38 patients).26

Two phase III studies of trastuzumab-DM1 are ongoing. One study tests the activity of trastuzumab-DM1 versus standard therapy with lapatinib–capcitabine as second-line therapy for patients with HER2-positive MBC. The other study tests docetaxel plus trastuzumab versus single-agent trastuzumab-DM1 as first-line therapy for HER2-positive MBC.

Inhibition of EGFR/HER1 phosphorylation by anti-EGFR agents does not always correlate with antitumor effects. This suggests that tumor proliferation may be controlled by alternate growth factors in the presence of EGFR inhibitors and that the antitumor activity of anti-EGFR agents may be improved by combining them with therapies targeting other signal transduction pathways.27 However, several studies in patients with breast cancer who were treated with these compounds as single agents showed disappointing results.28

**Dual EGFR and HER2 Inhibitors**

Interest in the role of EGFR in HER2-amplified tumors was renewed with the advent of dual TK inhibitors (TKIs) that interact with several EGFR members. Of these, lapatinib (Tykerb) is the agent that has been studied most extensively (Table 1). Other dual EGFR-HER2 inhibitors studied for breast cancer include cetuximab, canertinib, neratinib, and pertuzumab (Table 2).
Table 1. Phase II and III Trials of Lapatinib for Treatment of Breast Cancer

<table>
<thead>
<tr>
<th>Study and Author</th>
<th>No. of Patients</th>
<th>Type of Study</th>
<th>Patient Population</th>
<th>Lapatinib Dose (mg/d)</th>
<th>Combination Therapy</th>
<th>Response (%)</th>
<th>Patient Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PR</td>
<td>CR</td>
</tr>
<tr>
<td>Lapatinib single agent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.1</td>
<td>0</td>
</tr>
<tr>
<td>Blackwell et al²³</td>
<td>78</td>
<td>Phase II</td>
<td>HER2 positive and Tz refractory</td>
<td>1,250-1,500</td>
<td>—</td>
<td>5.1</td>
<td>0</td>
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<tr>
<td>Burstein et al²⁰</td>
<td>229</td>
<td>Phase II</td>
<td>A, T, and Cap refractory</td>
<td>1,500</td>
<td>HER2 positive and Tz refractory</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Arm A</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Arm B</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td>HER2 negative</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Gomez et al²¹</td>
<td>138</td>
<td>Phase II</td>
<td>HER2 positive; first-line treatment</td>
<td>1,500 once daily v 500 twice daily</td>
<td>—</td>
<td>24</td>
<td>0</td>
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Lapatinib in combination with chemotherapy, hormone therapy, and targeted therapy

<table>
<thead>
<tr>
<th>Study and Author</th>
<th>No. of Patients</th>
<th>Type of Study</th>
<th>Patient Population</th>
<th>Lapatinib Dose (mg/d)</th>
<th>Combination Therapy</th>
<th>Response (%)</th>
<th>Patient Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PR</td>
<td>CR</td>
</tr>
<tr>
<td>Geyer et al²⁵</td>
<td>324</td>
<td>Randomized, phase III</td>
<td>HER2 positive and A, T, and Cap refractory</td>
<td></td>
<td></td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Arm A</td>
<td>163</td>
<td></td>
<td></td>
<td></td>
<td>Cap 2,000 mg/d for 14 days</td>
<td>35</td>
<td>1</td>
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<tr>
<td>Arm B</td>
<td>161</td>
<td></td>
<td></td>
<td></td>
<td>cap 2,500 mg/d for 14 days</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Study and Author</td>
<td>No. of Patients</td>
<td>Type of Study</td>
<td>Patient Population</td>
<td>Lapatinib Dose (mg/d)</td>
<td>Combination Therapy</td>
<td>Response (%)</td>
<td>Patient Outcome</td>
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<td>-------------------------</td>
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<tr>
<td>Di Leo et al&lt;sup&gt;22&lt;/sup&gt;</td>
<td>579</td>
<td>Randomized, phase III</td>
<td>HER2 negative or HER2 UT</td>
<td>Arm A: 1,500</td>
<td>P 175 mg/m² every 3 weeks</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arm B: Pl</td>
<td>P 175 mg/m² every 3 weeks</td>
<td>23</td>
<td>2</td>
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<tr>
<td>Johnston et al&lt;sup&gt;23&lt;/sup&gt;</td>
<td>1,286</td>
<td>Randomized, phase III</td>
<td>Hormone receptor positive, HER2 negative, or hormone receptor positive, HER2 positive</td>
<td>Arm A: 1,500</td>
<td>Let 2.5 mg daily</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arm B: Pl</td>
<td>Overall, 219 of 1,286 patients were hormone receptor positive/HER2 positive. The PFS was 8.2 vs 3 months for L + Let vs Let alone (HR, 0.71; P = .019). Significant improvement in CB rate (29% vs 48%; P = .003) for the combination arm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Shaughnessy et al&lt;sup&gt;25&lt;/sup&gt;</td>
<td>296</td>
<td>Randomized, phase III</td>
<td>HER2 positive</td>
<td>Arm A: A, T, Cap, and Tz refractory</td>
<td>1,500</td>
<td>—</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arm B: Tz: 2 mg/kg weekly</td>
<td>after 4 mg/kg loading dose</td>
<td>1,000</td>
<td>25.2</td>
</tr>
</tbody>
</table>

Abbreviations: PR, partial response; CR, complete response; CB, clinical benefit; HER2, human epidermal growth factor receptor 2; Tz, trastuzumab, KPS, Karnofsky performance status; TTP, time to tumor progression; PFS, progression-free survival; AE, adverse event; A, anthracyclines; T, taxanes; Cap, capecitabine; L, lapatinib; HR, hazard ratio; UT, untested; EFS, event-free survival; OS, overall survival; ORR, overall response rate; P, paclitaxel; Pl, placebo; Let, letrozole; NA, not available; ER, estrogen receptor.
Lapatinib is a selective, reversible, dual EGFR-HER2 inhibitor. Lapatinib has a slower rate of dissociation from EGFR than erlotinib and gefitinib, which results in prolonged target-site downregulation.53

Lapatinib plus capecitabine was approved by the US Food and Drug Administration on March 13, 2007, for the treatment of patients with advanced or HER2-overexpressing MBC previously treated with an anthracycline, a taxane, and trastuzumab.5 In a phase I study of lapatinib in heavily pretreated patients with EGFR- and HER2-positive MBC, no DLT was found54; the most common adverse effects were diarrhea and rash, and there were no grade 4 toxic effects. Four of 59 evaluable patients with trastuzumab-resistant disease, including two with inflammatory breast cancer, had a PR, and all of these patients had high expression of activated phosphorylated HER2.

The pivotal trial that led to regulatory approval of lapatinib showed that lapatinib plus capecitabine increased PFS compared with capecitabine alone in patients with locally advanced or metastatic HER2-positive breast cancer not controlled by previous treatment with trastuzumab.55

### Table 2. Dual EGFR and HER2 Inhibitors

<table>
<thead>
<tr>
<th>Agent</th>
<th>Drug Class</th>
<th>Mechanism of Action</th>
<th>Study Comments</th>
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<tbody>
<tr>
<td>Lapatinib</td>
<td>TKI</td>
<td>Irreversible inhibitor of EGFR and HER2</td>
<td>A phase II study of lapatinib plus Tz in patients with HER2-positive breast cancer cell lines, Tz plus pertuzumab increased apoptosis and cell growth arrest compared with Tz alone. A two-stage, phase II study of pertuzumab plus Tz in patients with previously treated (including with adjuvant Tz), HER2-positive MBC showed that, of 86 patients evaluable for response, five experienced CR, 11 had PR, and 17 had SD for approximately 6 months. Thirty-three (50%) of 66 patients had CB. There were no clinical cardiac events and no occurrences of decrease in LVEF greater than 10%.</td>
</tr>
<tr>
<td>Canertinib</td>
<td>TKI</td>
<td>Irreversible inhibitor of all EGFR family</td>
<td>Preclinical activity was documented in mouse xenografts model, including breast cancer. A phase I study in heavily pretreated patients on canertinib, including patients with breast cancer, showed MTD doses of 225 mg three times a week and 280 mg with a 7-day on, 7-day off schedule. In phase I and phase II studies, the most common adverse effects were gastrointestinal toxicity and rash. Compared with oral delivery, intravenous delivery produced fewer gastrointestinal adverse events and increased bioavailability three-fold. A phase I study of canertinib plus docetaxel in patients with advanced solid tumors resulted in a recommended phase II dose of canertinib 50 mg/d plus docetaxel 75 mg/m².</td>
</tr>
<tr>
<td>Neratinib</td>
<td>TKI</td>
<td>Irreversible inhibitor of EGFR and HER2</td>
<td>In phase I/I study in MBC patients who were HER2 positive and who experienced progression on Tz therapy, 45 patients were treated with neratinib 160 mg or 240 mg daily plus Tz. Among 33 evaluable patients, the objective RR was 27% (95% CI, 13% to 46%), and median PFS was 19 weeks (95% CI, 15 to 32 weeks). In a phase II study, patients with stage IIIB to IV, HER2-positive MBC were assigned to arm A (n = 65) if they had received Tz and to arm B (n = 66) if they had not received Tz or another HER2-targeting drug, and patients received neratinib 240 mg daily. The primary end point, median PFS, was 23 weeks (95% CI, 16 to 39 weeks) for arm A and 40 weeks (95% CI, 32 to 55 weeks) for arm B. RR for arms A and B were 26% and 56%, respectively, and CB rates were 36% and 68%, respectively. One fourth of the patients required dose reductions; grade 3 diarrhea was seen in five patients (19%).</td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>mAb</td>
<td>Bind different HER2 epitope of the HER2 than Tz, blocking heterodimerization of HER2 with EGFR and ErbB3</td>
<td>In HER2-positive breast cancer cell lines, Tz plus pertuzumab increased apoptosis and cell growth arrest compared with Tz alone. A two-stage, phase II study of pertuzumab plus Tz in patients with previously treated (including with adjuvant Tz), HER2-positive MBC showed that, of 86 patients evaluable for response, five experienced CR, 11 had PR, and 17 had SD for approximately 6 months. Thirty-three (50%) of 66 patients had CB. There were no clinical cardiac events and no occurrences of decrease in LVEF greater than 10%. Patients are currently being enrolled on a phase III study of pertuzumab plus Tz as first-line treatment for HER2-positive MBC (CLEOPATRA study).</td>
</tr>
</tbody>
</table>

Abbreviations: mAb, monoclonal antibody; EGFR, epidermal growth factor receptor; P, paclitaxel; MBC, metastatic breast cancer; DLT, dose-limiting toxicity; SD, stable disease; PR, progressive disease; TRN, triple-receptor negative; CP, carboplatin; RR, response rate; CB, clinical benefit; PFS, progression-free survival; OS, overall survival; TKI, tyrosine kinase inhibitor; HER2, human epidermal growth factor receptor 2; Tz, trastuzumab; CR, complete response; PR, partial response; LVEF, left ventricular ejection fraction; CLEOPATRA, Clinical Evaluation of Pertuzumab and Trastuzumab.
anthracyclines, taxanes, and trastuzumab. The study was closed prematurely, because the first interim analysis showed that the addition of lapatinib was associated with a 51% reduction in the risk of disease progression. The median times to progression for patients treated with lapatinib plus capecitabine and for patients treated with capecitabine plus placebo were 8.4 months and 4.4 months, respectively (hazard ratio, 0.49; 95% CI, 0.34 to 0.71; P < .001; Appendix Fig A1, online only). Eleven patients in the capecitabine group had progressive CNS metastasis compared with four in the combination-therapy group (P = .10). One third of women with HER2-positive MBC who receive trastuzumab developed CNS metastasis. Small molecules, such as lapatinib, can cross the blood-brain barrier. In a recent phase II study of patients with HER2-positive breast cancer and brain metastasis, rates of objective response, defined as ≥ 50% reduction in the volume of the brain lesion(s), were 6% for patients treated with lapatinib and 20% for patients treated with lapatinib and capecitabine; furthermore, 21% of the patients treated with lapatinib alone and 40% of the patients treated with combination therapy experienced at least a 20% volumetric reduction in their CNS lesion(s).

Concerns have been voiced about the potential cardiotoxicity of lapatinib, but a recent pooled analysis of 3,689 lapatinib-treated patients revealed low rates of cardiac toxic effects. These effects were mostly asymptomatic decreases in left cardiac ejection fraction.

Preclinical studies showed a synergistic interaction between lapatinib and trastuzumab in HER2-overexpressing breast cancer cell lines and tumor xenografts. Preliminary results of a randomized, phase III trial of lapatinib with or without trastuzumab in patients with heavily pretreated HER2-positive MBC demonstrated synergy and improved median PFS with combination therapy. Ongoing are a large trial of lapatinib plus trastuzumab as adjuvant therapy (the Adjuvant Lapatinib and Trastuzumab Treatment Optimization [ALTTO] trial) and a small trial of lapatinib plus trastuzumab as primary systemic therapy (the Neo-ALTTO trial) in patients with HER2-positive, early-stage breast cancer.

LAPATINIB PLUS HORMONAL AGENTS

Evidence is accumulating that signaling interplay between the estrogen receptor, HER2, EGFR, and insulin-like growth factor (IGF) 1 receptor plays a role in the acquired resistance to hormonal therapies. In a preclinical model, lapatinib restored tamoxifen sensitivity in hormone-receptor-positive, tamoxifen-resistant breast cancer. Several studies investigating lapatinib plus hormonal agents are planned.

In the EGF30008 trial, a phase III study of letrozole with or without lapatinib in postmenopausal patients with hormone-receptor-positive, HER2-positive MBC, the combination therapy resulted in a 29% reduction in the risk of disease progression (P = .019), and the median PFS improved from 3.0 to 8.2 months. Ongoing is a large, European, phase II study of letrozole with or without lapatinib as neoadjuvant therapy in patients with hormone-sensitive, HER2-negative, operable breast cancer (the LET-LOB study). Lapatinib is also active in patients with newly diagnosed inflammatory breast cancer, both alone and with paclitaxel.

PERTUZUMAB

The discovery of the crucial role of ERBB3 in mediating signaling with different dimers and blocking ERBB2-dependent signaling through the phosphatidylinositol-3-kinase (PI3K) – Akt pathways provides an excellent opportunity for the development of TKIs with specific activity against ERBB3. Because ERBB3 lacks intrinsic kinase activity, though, the generation of specific HER3-directed TKIs is challenging. Pertuzumab is an ERBB2 antibody that inhibits ERBB3 signaling by blocking ligand-induced HER2-to-HER3 heterodimerization. Preclinical observation in several breast cancer cell lines suggested that interfering with the ERBB3 component may be more relevant than inhibition of EGFR in HER2-amplified breast cancer cell lines. In patients with ovarian cancer, high levels of ERBB3 correlated with shorter overall survival than ERBB2 overexpression.

NERATINIB

Recent preliminary data showed impressive antitumor activity in patients with trastuzumab-pretreated, HER2-amplified breast cancer after treatment with neratinib, a highly selective irreversible inhibitor of EGFR and ERBB2. Mature data are awaited, and more studies are underway.

NEW-GENERATION ANTI-HER2 TYROSINE KINASES

A new generation of anti–HER2 TKs is being developed. Among these new agents are EKB-569 and BIBW 2992, which are currently being studied in clinical trials. The bispecific (ertumaxomab) and trispecific antibodies that target ERBB2 are also under investigation.

The therapeutic armamentarium against the EGFR family, especially HER2-positive disease, has grown in the past decade. Results from the clinical trials highlight the potential of combination anti-HER2 therapies that might be superior to single-agent strategies. For instance, combination of both anti-HER2 therapies–lapatinib and trastuzumab–in patients in whom trastuzumab failed is superior to lapatinib alone. Coexpression of both estrogen receptor and HER2 are reported in approximately 50% of patients with breast cancer; preclinical data suggest that HER2 overexpression confers intrinsic resistance to hormonal therapy. However, new clinical evidence reveals that the combination of anti-HER2 and hormonal therapy could be considered the treatment of choice at this time.

VEGF INHIBITORS

Angiogenesis plays an essential role in breast cancer development, invasion, and metastasis. Agents that block the VEGF pathway have been shown to effectively inhibit tumor angiogenesis and growth in preclinical tumor models (Fig 2; Appendix Fig A2, online only). Studies in early-stage breast cancer show that elevated VEGF expression is associated with decreased relapse-free survival and overall survival in patients with both lymph-node–positive and lymph-node–negative disease. Several drugs that target VEGF ligands or receptors have now emerged into the clinic (Appendix Table A2, onlineonly).
bevacizumab was added to chemotherapy. Bevacizumab as neoadjuvant therapy is under investigation in a large study by the National Surgical Adjuvant Breast and Bowel Project (NSABP-B40), and bevacizumab as maintenance therapy in patients with TRN breast cancer is being investigated in the Bevacizumab Adjuvant Therapy in Triple-Negative Breast Cancer (BEATRICE) trial. There are two large, ongoing, randomized, phase III trials of bevacizumab as adjuvant therapy: ECOG 5103, which compares chemotherapy versus chemotherapy plus bevacizumab, and the Bevacizumab and Trastuzumab Adjuvant Therapy (BETH) trial, which compares chemotherapy with doceletaxel, carboplatin, and trastuzumab with or without bevacizumab for HER2-amplified breast cancer.

Aflibercept

Aflibercept is a soluble decoy receptor protein that consists of a fusion of the second immunoglobulin domain of the VEGF receptor-1 (VEGFR-1) and the third immunoglobulin domain of the human VEGFR-2 with the constant region of human immunoglobulin G1. Aflibercept recognizes the entire VEGF family that binds to VEGFR-1 and VEGFR-2, including placental growth factor, and possesses higher affinity for VEGF than bevacizumab in vitro. Aflibercept potently inhibited tumor growth, metastasis formation, and ascites formation in several murine tumor models.

Several monoclonal antibodies, including HuMV833, IMC-1121B, and IMC-18F1, have been designed to target selected portions of VEGFR. These agents are under investigation in clinical trials.

Sunitinib

Sunitinib malate (Sutent; Pfizer, New York, NY) is an oral TKI that targets several receptor TKs, including VEGFR-1, VEGFR-2, and VEGFR-3; platelet-derived growth factor receptor-α (PDGFR-α) and PDGFR-β; c-Kit; and colony-stimulating factor-1 receptor. In a phase I study in which patients with solid tumors received sunitinib 15 to 59 mg/m², six of 28 patients had a PR. The most common adverse effects were fatigue, hypertension, and skin manifestations. In a phase II trial in 64 patients with MBC previously treated with anthracyclines and taxanes who received sunitinib at a starting dose of 50 mg once daily for 4 weeks of a 6-week cycle, seven patients (11%) had a PR,
and three patients had stable disease for more than 6 months, for an overall CB rate of 16%. Objective responses occurred in three of 20 patients with TRN tumors and in three of 12 patients with HER2-positive tumors. Grade 3 fatigue and hand-foot syndrome occurred in 14% and 9% of patients, respectively; one third of patients experienced grade 3 neutropenia. In a phase II, randomized study, 46 patients with TRN tumors and in three of 12 patients with HER2-negative MBC were randomly assigned to receive paclitaxel 90 mg/m² weekly and bevacizumab 10 mg/kg every 2 weeks with or without sunitinib 25 mg daily for 21 days as first-line chemotherapy. Sunitinib was associated with high rates of dose modification and treatment discontinuation because of toxic effects—including neutropenia, febrile neutropenia, and fatigue—that led to closure of the study.

Sunitinib also was studied in combination with metronomic dosing of cyclophosphamide and methotrexate in patients with MBC. Fifteen patients were treated in three sunitinib dose cohorts: 12.5 mg/d, 25 mg/d, and 37.5 mg/d. Three patients developed grade 3 neutropenia, and five developed mucositis. One patient had a PR at

<table>
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<th>Trial</th>
<th>No. of Patients</th>
<th>Patient Population</th>
<th>Bevacizumab</th>
<th>Combination Therapy</th>
<th>End Point</th>
<th>Benefit in Anti-VEGF Therapy</th>
<th>Study Primary Results</th>
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</thead>
<tbody>
<tr>
<td>AVF2119</td>
<td>462</td>
<td>PT MBC</td>
<td>15 mg/kg every 3 weeks</td>
<td>Cap 2,500 mg/m²² from day 1 to day 14</td>
<td>PFS</td>
<td>No</td>
<td>Bev and Cap significantly increased the ORR compared with Cap as a single agent (9.1% v 19.8%; P = .001), but not PFS (4.2 v 4.0 months; HR, 0.98). No significant differences were found in the incidence of diarrhea, hand-foot syndrome, and serious bleeding episodes between treatment groups.</td>
</tr>
<tr>
<td>ECOG 2100</td>
<td>722</td>
<td>FL MBC</td>
<td>10 mg/kg every 2 weeks</td>
<td>P 90 mg/m²² on days 1, 8, and 15</td>
<td>PFS</td>
<td>Yes</td>
<td>Bev and P significantly prolonged PFS compared with P alone (median, 11.8 v 5.9 months; HR for progression, 0.60; P = &lt; .001) and increased ORR (36.9% v 21.2%). No differences in OS between the two groups (median 26.7 v 25.5 months; HR, 0.88; P = .16). AE: grade 3 or 4 hypertension (14.8% v 0%; P = &lt; .001), proteinuria (0.6% v 0%; P = &lt; .001), headache (2.2% v 0%; P = &lt; .008), and cerebrovascular ischemia (1.9% v 0%; P = .02) were more common in patients receiving the combination treatment.</td>
</tr>
<tr>
<td>AVADO</td>
<td>736</td>
<td>FL MBC</td>
<td>7.5 mg/kg every 3 weeks or 15 mg/kg every 3 weeks</td>
<td>D 100 mg/m² every 3 weeks</td>
<td>PFS</td>
<td>Yes</td>
<td>In unstratified analysis, patients receiving Bev had significantly longer PFS compared with the D monotherapy group (Bev at 7.5 mg/kg: median PFS, 8.7 v 8.0 months; HR, 0.79; P = .0318; Bev at 15 mg/kg: median PFS, 8.8 v 8.0 months; HR, 0.72; P = .0099). ORR improved with the addition of Bev. Bev 7.5 mg/kg, 55% v 44% (P = .0295); Bev 15 mg/kg 63% v 44% (P &lt; .001). The study was not powered to find differences in OS.</td>
</tr>
<tr>
<td>RIBBON1</td>
<td>1,237</td>
<td>FL MBC</td>
<td>15 mg/kg every 3 weeks</td>
<td>Cap, taxanes (Nab-Pac and DI), anthracycline</td>
<td>PFS</td>
<td>Yes</td>
<td>The median follow up was 15.6 months in the Cap cohort and 19.2 months in the taxane and anthracycline cohort. The addition of Bev to Cap, taxanes, or anthracycline-based chemotherapy resulted in statistically significant improvement in PFS.</td>
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<tr>
<td>MO19391</td>
<td>2,027</td>
<td>HER2 negative MBC or HER2 positive if previous Tz</td>
<td>10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks</td>
<td>Taxane-based chemotherapy</td>
<td>Safety</td>
<td>Yes</td>
<td>Median follow up was 7.4 months; approximately 75% of patients received taxanes, and 25% were treated with non-taxane regimens (Cap and VNR). Safety and efficacy of Bev plus D or P was similar to results of ECOG2100 and AVADO.</td>
</tr>
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</table>

Abbreviations: VEGF, vascular endothelial growth factor; PT, pretreated; MBC, metastatic breast cancer; Cap, capecitabine; PFS, progression-free survival; Bev, bevacizumab; ORR, overall response rate; HR, hazard ratio; ECOG2100, Eastern Cooperative Oncology Group trial 2100; FL, first line; P, paclitaxel; OS, overall survival; AE, adverse event; AVADO, Avastin and Docetaxel; D, docetaxel; RIBBON1, Regimens in Bevacizumab for Breast Cancer; Nab-Pac, Nab-paclitaxel; HER2, human epidermal growth factor receptor 2; Tz, trastuzumab; VNR, vinorelbine. Currently enrolling patients.
week 14, and one patient had stable disease for 47 weeks. Enrollment continues.

**Sorafenib**

Studies of sorafenib ( Nexavar; Bayer/Onyx Pharmaceuticals, West Haven, CT) have mainly focused on optimizing dosing to maximize activity against Ras. Preclinical studies, daily sorafenib significantly inhibited tumor growth and microvessel density in an MDA-MB-231 breast cancer xenograft model. A phase I study showed a favorable toxicity profile of sorafenib 400 mg twice daily in patients with advanced solid tumors.

In a two-stage, phase II study of sorafenib 300 mg twice daily in patients with MBC refractory to anthracyclines and taxanes, the median number of cycles was 2, and dose reductions were necessary because of dermatitis/skin rash (n = 3), hand-foot syndrome (n = 2), and hypertension (n = 1). One of 20 patients eligible for efficacy evaluation had a PR that lasted 3.6 months. The study was closed after the first stage because of lack of sufficient response.

**Vandetanib**

Vandetanib (Zactima; AstraZeneca) is a potent inhibitor of kinase insert domain-containing receptor (VEGFR-2), VEGFR-3, and EGFR/HER1. A phase I dose-finding study established a dose of 300 mg daily. A phase II study in 46 patients with MBC refractory to anthracyclines and taxanes showed no objective responses. The authors hypothesized that the lack of activity could be related to inadequate blood concentration of vandetanib. Most patients achieved a plasma concentration greater than the 50% inhibitory concentration; however, adverse effects commonly seen with VEGF inhibitors (eg, hypertension, headache, thrombosis) and EGF inhibitors (eg, severe rash) were not seen.

**Vatalanib**

Vatalanib is an oral inhibitor of VEGFR-1, VEGFR-2, and VEGFR-3 and of other related kinases. A phase I study in patients with advanced solid tumors established that the maximum tolerated dose was 750 mg twice daily, whereas the biologically active dose was greater than 1,000 mg twice daily. The Hoosier Oncology Group recently finished accruing patients for a phase I/II study of vatalanib plus trastuzumab in patients with newly diagnosed, HER2-overexpressing MBC.

**Axitinib**

Axitinib is a potent small-molecule TKI of all known VEGFRs, PDGFR-β, and c-Kit. The initial phase I study in patients with solid tumors showed a 10% PR rate. Fewer than 10% of the patients experienced grade 3 or 4 toxic effects; hypertension was the most common adverse effect and was reported in 22 patients (61%), 11 of whom had grade 3 or 4 hypertension. The incidence and severity of hypertension were dose related. Other DLTs observed were stomatitis (6%) and hemoptysis (3%).

In 2007, preliminary findings were reported from a phase II multicenter, randomized, double-blind, placebo-controlled trial of docetaxel (80 mg/m² every 3 weeks) alone or with axitinib (5 mg twice daily) in 168 patients with chemotherapy-naïve MBC. The overall response rate was 40% with docetaxel plus axitinib and was 23% with docetaxel plus placebo (P = .038); the median time to treatment failure was 9 months with docetaxel plus axitinib and was 6.3 months for docetaxel plus placebo (P = .012). Grades 3 and 4 adverse effects were more common with axitinib: febrile neutropenia (16% v 7%), fatigue (13% v 5%), stomatitis (13% v 2%), diarrhea (11% v 0%), and hypertension (5% v 2%).

The Ras superfamily of GTPases act as crucial regulatory switches coordinating a variety of biologic functions. These proteins are classified in five families: Ras, Rho, Rab, Sari1/Arf, and Ran. Although fewer than 5% of breast cancers have ras mutations, hyperactivation of the Ras protein in breast cancer has been described. Overexpression of Rho was associated with locoregional and distant metastasis of breast cancer and also inflammatory breast cancer.

Tipifarnib (Zarnestra; Johnson & Johnson, New Brunswick, NJ), a farnesyltransferase inhibitor, inhibited the growth of MCF-7 breast cancer cell xenografts in a dose-dependent manner. In a phase I trial, single-agent tipifarnib was administered at doses up to 1,300 mg twice daily for 5 days every 2 weeks without significant toxicity. The authors recommended that the tipifarnib dose for phase II trials be 500 mg twice daily for 5 consecutive days followed by 9 days of rest.

In a phase II study of tipifarnib in patients with hormone-sensitive MBC who experienced progression during second-line hormonal therapy, 10% of patients had a PR, and 25% had CB. The main adverse effects were neutropenia, thrombocytopenia, and neurotoxic effects. In another study, tipifarnib was combined with dose-dense doxorubicin and cyclophosphamide as neoadjuvant therapy for patients with locally advanced breast cancer; after four cycles, patients underwent surgery. Five of 32 patients had at least 50% farnesyltransferase inhibition in the primary tumor, as revealed by serial biopsies during treatment, and seven of 21 patients had a pathologic complete response. These data are interesting, because pathologic complete response occurred in patients with estrogen–receptor–positive tumors.

In a randomized, phase II study in 120 patients with MBC who experienced antiestrogen therapy failure, addition of tipifarnib to letrozole did not improve the objective response rate. However, in another phase II study in patients with no prior therapy for MBC, tipifarnib combined with fulvestrant resulted in a CB rate of 51.6%.

The PI3K signaling pathway is crucial to many aspects of key cellular functions, including growth, proliferation, survival, angiogenesis, and motility. Recent studies indicate that, in patients with cancer, amplification, mutation, and translocation that result in activation are more common in the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway than in any other pathway. Activating mutation of PI3K has been described in approximately 40% of primary breast tumors, which suggests the importance of PI3K in breast cancer tumorigenesis. Three mTOR antagonists are being studied for breast cancer treatment: everolimus, a mammalian target of rapamycin inhibitor with better oral availability than sirolimus; temsirolimus, a water-soluble ester of sirolimus; and deforolimus (AP23573), a non-rapamycin analog prodrug that has been tested in phase I and II.
clinical trials and that shows promising results in several tumor types, including sarcoma. All three agents have shown activity against breast cancer in preclinical studies. Everolimus and temsirolimus showed good adverse effect profiles.

**Everolimus**

Everolimus (G cetican; Novartis Pharma AG, Basel, Switzerland) was developed in an attempt to improve the pharmacokinetic characteristics of sirolimus, particularly to increase oral bioavailability. In a phase II, randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with operable estrogen-receptor–positive breast cancer, everolimus plus letrozole was associated with a significantly higher clinical response rate (68% v 59%; \(P = .0616\)).

**Temsirolimus**

Temsirolimus (Torisel; Wyeth, Philadelphia, PA) is a water-soluble ester of sirolimus with antitumor activity in preclinical breast cancer models. In a phase I study in patients with advanced malignancies treated with weekly intravenous temsirolimus (7.5 to 220 mg/m\(^2\)), the DLT was thrombocytopenia.

In a phase II study in previously treated patients with locally advanced breast cancer or MBC treated with weekly intravenous temsirolimus (75 mg or 250 mg), 13.8% of patients had CB. The most common adverse effects were mucositis, maculopapular rash, and nausea. Preliminary results of a large, phase II study of temsirolimus plus letrozole or letrozole alone showed similar rates of CB for the two approaches (82% and 83% for continuous and intermittent temsirolimus, respectively, and 79% for letrozole alone) but suggested that PFS might be longer for combination therapy.

In a phase III study, more than 1,200 postmenopausal patients with estrogen-receptor–positive MBC suitable for first-line therapy were randomly assigned to letrozole with or without temsirolimus. The trial was terminated early after interim analysis demonstrated a lack of additional benefit with the combination therapy. Studies of temsirolimus in combination with other drugs are ongoing.

**INSULIN-LIKE GROWTH FACTOR INHIBITORS**

The IGF system involves a complex regulatory network composed of two receptors, two ligands, and IGF-binding proteins. Several monoclonal antibodies (CP-751,856, AMG 479, and IMC-A12) are in early clinical development in the treatment of breast cancer.

**POLY (ADP-RIBOSE) POLYMERASE 1 INHIBITORS**

Poly (ADP-ribose) polymerase 1 (PARP-1) is a critical enzyme in cell proliferation and DNA repair. Multiple PARP-1 inhibitors have been tested preclinically as potentiators of chemotherapy and radiotherapy. A preliminary analysis of a randomized, phase II study of gemcitabine plus carboplatin with or without the PARP-1 inhibitor BSI-201 in patients with TRN MBC showed the high objective response rate and longer PFS and overall survival with BSI-201 (Fig 3; Appendix Fig A3, online only).

Olaparib (AZD2281) is a novel PARP inhibitor with significant activity in patients with breast, ovarian, and prostate cancer with BRCA1 or BRCA2 mutation. A phase I study showed that 12 of 19 patients had CB, and nine patients had PR by Response Evaluation Criteria in Solid Tumors (RECIST). A preliminary report of a single-arm, phase II study in patients with BRCA-deficient breast cancer treated with olaparib was recently published. Nineteen of 54 patients who received 400 mg daily of olaparib had PR by RECIST; 19% of patients experienced grade 3 or 4 toxic effects, including fatigue (11%), nausea (2%), and vomiting (5.5%). Several phase II studies of other PARP inhibitors (ie, ABT-888, AGO14699, and MK4827) are underway.

**FUTURE DIRECTIONS**

The past decade has also been one of dramatic changes in breast cancer treatment, including increasing use of targeted therapy. However, despite great enthusiasm for targeted therapy, these agents have exhibited only anecdotal or modest activity when used as single agents in unselected patients. In addition, selection of patients for targeted therapy remains a challenge, because we lack reliable biomarkers to predict activity for most of the targeted agents.

The development of new drugs in oncology faces multiple challenges in this new molecular era. The final major contribution to the transformation of breast cancer treatment has been not a technical or pharmacologic revolution but rather a transformation in the way we think about the disease and its treatment. Continued application of old paradigms of drug evaluation (on the basis of response rates and toxicity) to new targeted therapies may be inappropriate, because neither tumor response nor toxicity is a useful surrogate for dose selection or efficacy. We need a better understanding of the molecular...
biology of signaling pathways, and we need to discover new biomarkers that we can use to select the optimal dose of targeted agents for phase II clinical studies.

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