Growth Factor Receptors in Breast Cancer: Potential for Therapeutic Intervention

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ABSTRACT

Increased expression and activation of receptor tyrosine kinases occurs frequently in human breast carcinomas. Several therapies targeting these receptors are currently in clinical trials. Therapeutic strategies include blockade of individual receptors with monoclonal antibodies and inhibition of tyrosine kinase function. Trastuzumab is the first of these biologic therapies to be approved for patients with human epidermal growth factor receptor 2 (HER2)-overexpressing metastatic breast cancer. Novel trastuzumab-based combinations are being investigated in patients with advanced breast cancer. Large clinical trials have also been launched in the adjuvant setting. Small molecules that inhibit specific tyrosine kinases (e.g., epidermal growth factor receptor, HER2) are in phase I and phase II clinical trials. Other growth-factor-targeted drugs that have reached clinical development include STI571 and antibodies directed at the insulin-like growth factor pathway. Biologic therapies directed against these important receptors are promising. In this review we discuss challenges and opportunities for the development of growth-factor-targeted approaches for the treatment of breast cancer. The Oncologist 2003; 8:5-17

INTRODUCTION

Traditional cancer therapeutics have relied heavily upon the ability to inhibit DNA replication or cell division [1]. While this approach has proven effective in some patients, lack of tumor-cell selectivity has limited response rates and complicated treatment with numerous adverse effects. Thus, the current goal of anticancer drug design is to directly target specific molecular lesions found in tumor cells in the hope of improving cancer cure rates and reducing cytotoxicity in normal cells. The efficacy of this “targeted” approach is best illustrated by therapies that inhibit estrogen receptor (ER) function or synthesis of estrogen. The ability of the selective ER modulator tamoxifen to inhibit growth of and prevent ER-positive breast cancers demonstrates that specific components of individual growth factor pathways can be directly targeted to successfully treat breast cancer [2].

Breast cancer cells require activated growth factor receptors to proliferate, invade, and metastasize in experimental models. Overexpression of growth factor receptors has been associated with a poor clinical outcome in breast cancer patients. Biological therapy directed against growth factor receptor pathways is being pursued on a variety of fronts. This review focuses on the available preclinical data and on clinical research efforts to develop growth factor receptor inhibitors into novel therapeutics for breast cancer.

EPIDERMAL-GROWTH-FACTOR-RECEPTOR-TARGETED THERAPY

Dr. Stanley Cohen first identified the epidermal growth factor (EGF) peptide and its 170-kD receptor (EGFR), both of which were later found to contribute to the unregulated proliferation of cancer cells through an autocrine growth-promoting mechanism [3-5]. The EGFR was first linked to
human cancer when homology was identified with the viral oncogene \( v-erbB \), and its tyrosine kinase function was found to be similar to that of the \( Src \) oncogene [6-10]. Overexpression of wild-type EGFR or expression of a truncated form of EGFR (EGFR vIII) is found in some breast cancers (Table 1). Therapies targeting the EGFR are likely to be beneficial in this subset of tumors. Strategies including antireceptor antibodies, tyrosine kinase inhibitors, ligand-toxin conjugates, and receptor antisense molecules have been explored, as outlined in Figure 1. As antireceptor antibodies and tyrosine kinase inhibitors are currently in clinical trials, we focus on experiences related to these strategies.

### Anti-EGFR Antibodies

Several murine monoclonal antibodies (mAbs) targeted toward the EGFR extracellular region have been produced. These antibodies compete with ligands for receptor binding, blocking autocrine and paracrine growth factor loops, and induce receptor dimerization and downregulation [11, 12]. Furthermore, receptor blockade by antibodies inhibits downstream signaling, producing various molecular and biological effects that may inhibit the growth of EGFR- and human epidermal growth factor receptor 2 (HER2)-expressing breast cancer cells. However, the use of these murine mAbs has been limited by their ability to produce immune responses characterized by production of human anti-mouse antibodies. Thus, to reduce immunogenicity, human-mouse chimeric and fully humanized antibodies have been generated [13].

**IMC-C225**

IMC-C225 (cetuximab; ImClone Systems; New York, NY) is a chimeric mAb directed against an extracellular epitope of the EGFR. IMC-C225 competitively inhibits binding of the EGFR ligands EGF and transforming growth factor-\( \alpha \) due to a 10-fold higher affinity for the receptor. Preclinical evidence of anticancer activity is summarized in Table 2. While in vitro data suggest that IMC-C225 is cytostatic [13, 14], MDA468 breast cancer xenografts regressed when treated with IMC-C225, suggesting that additional antitumor mechanisms function in vivo [13, 15]. These mechanisms may include reduced angiogenesis, decreased metastasis, or immunocytotoxicity [11]. Phase I/II studies of IMC-C225 in EGFR-overexpressing cancers achieved stable disease when administered as a single agent and some partial remissions.
when combined with cisplatin or irinotecan in head and neck, colorectal, and non-small cell lung cancers. No major organ toxicities were documented, but acneiform rash and folliculitis were commonly observed. Across all clinical trials, immunogenic responses against the murine portion of the chimeric mAb were reported in only 4% of patients [16]. IMC-C225 has also demonstrated increased response to radiation therapy in head and neck tumors [17]. Several phase II/III trials of IMC-C225 are currently in progress for various solid tumors (Table 3).

**ABX-EGF**

Fully humanized antibodies eliminate the immunogenic effect of mouse and chimeric mouse-human antibodies and can be produced through XenoMouse® technology (Abgenix; Fremont, CA). Through this method, mouse antibody genes are inactivated and replaced with genes encoding human antibodies. Hence, mice immunized with EGFR will produce fully humanized antireceptor antibodies [18, 19]. ABX-EGF was produced by XenoMouse® technology and possesses high affinity and specificity for the EGFR. Complete eradication of A431 tumor xenografts occurred upon treatment with ABX-EGF in the absence of chemotherapy [20, 21]. Furthermore, ABX-EGF suppressed growth of MDA468 xenografts, demonstrating its potential efficacy against breast tumors [20, 21]. Cooperation of ABX-EGF with chemotherapeutic agents such as doxorubicin has also been demonstrated. Interestingly, ABX-EGF prevented solid tumor formation in nude mice injected with cancer cells, suggesting development of this antibody as a preventive agent [21]. Phase I clinical trials are currently examining ABX-EGF in solid tumors.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Molecular and cellular effects</th>
<th>Breast cancer cells examined</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib mesylate (STI571)</td>
<td>1. competitive ATP inhibitor; 2. antitumor activity; prevents metastatic disease in vivo</td>
<td>MDA468, MCF10A, ZR75-1, MDA68</td>
<td>[108]</td>
</tr>
<tr>
<td>IMC-C225</td>
<td>1. downregulates the EGFR; 2. G1 arrest: induces p27kip1, inhibits cdk2, cyclin D1; 3. apoptosis: inactivates Bcl-2; 4. synergy with doxorubicin</td>
<td>MDA468</td>
<td>[14, 144, 145]</td>
</tr>
<tr>
<td>ABX-EGF</td>
<td>1. downregulates the EGFR; 2. growth inhibition at fivefold lower concentration than IMC-C225; 3. potentially nontoxic to normal cells: arrests cells with minimum 17,000 EGF receptors, normal breast cells have 10,000 receptors</td>
<td>MDA468</td>
<td>[20, 21]</td>
</tr>
<tr>
<td>ZD1839</td>
<td>1. G1 arrest: induces p27kip1, inhibits cdk2; 2. angiogenic inhibitor; 3. additive growth inhibition with taxanes, doxorubicin; 4. sensitizes to hormone therapy; 5. synergy with trastuzumab; 6. in vivo: reduced proliferation, increased apoptosis, tumor regression, inhibition of EGFR/HER2+ tumors</td>
<td>BT474, ZR75-1, MCF-7, MDA468, MCF10A-Ha-Ras, MCF-7 ADR, MDA231</td>
<td>[146-151]</td>
</tr>
<tr>
<td>OSI-774</td>
<td>1. G1 arrest: induces p27kip1, inhibits cdk2; 2. apoptosis; 3. synergy with doxorubicin; 4. tumor regression in vivo</td>
<td>MDA468</td>
<td>[11, 23, 25, 152]</td>
</tr>
<tr>
<td>CI-1033</td>
<td>1. pan-Her TKI; 2. blocks EGFR vIII kinase; 3. enhances ubiquitin-mediated degradation of EGFR, HER2; 4. synergy with gemcitabine, ionizing radiation; 5. significant tumor regression in vivo</td>
<td>T47D, MDA453</td>
<td>[22, 153-155]</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>1. downregulates HER2; 2. G1 arrest, apoptosis; 3. angiogenic inhibitor; 4. antibody-dependent cellular cytotoxicity; 5. synergy with doxorubicin, taxanes, vinorelbine, flavopiridol</td>
<td>SKBR3, BT474</td>
<td>[64, 67, 68, 156]</td>
</tr>
<tr>
<td>E1A</td>
<td>1. transcriptional repression of HER2 promoter; 2. reduced tumor formation and increased survival rate</td>
<td>MDA361, SKBR3</td>
<td>[97, 98]</td>
</tr>
<tr>
<td>2C4</td>
<td>1. sterically hinders HER2 dimerization and signaling in low and high HER2-expressing breast cancers; 2. inhibits heregulin- and EGF-mediated tumor growth</td>
<td>MCF7, T47D, ZR751, SKBR3 cells, BT474, and MCF-7 xenografts</td>
<td>[157]</td>
</tr>
</tbody>
</table>
EGFR Tyrosine Kinase Inhibitors

As tyrosine kinase activity is required for EGFR-mediated tumorigenicity, therapies that ablate this function are currently being tested in clinical trials. Mutations in the EGFR ATP-binding site were shown to eliminate receptor kinase activity and prevent cellular transformation. Thus, small molecule tyrosine kinase inhibitors (TKIs) that competitively block ATP binding were designed as potential anticancer agents. Importantly, since these agents target an intracellular region of the EGFR, they could potentially inhibit the highly tumorigenic EGFR mutant vIII, which is a truncated receptor frequently found in breast cancer and may be inaccessible to mAbs [22]. Quinazoline compounds represent a class of competitive inhibitors of the ATP-binding site that are orally active, potent, and selective tyrosine kinase inhibitors [23]. Among the most widely examined thus far are the EGFR-specific ZD1839 and OSI-774 and an inhibitor of all four Her family receptors (called a pan-Her inhibitor), CI-1033.

ZD1839

ZD1839 (Iressa™; AstraZeneca; Macclesfield, UK) reversibly inhibits the EGFR and its downstream signaling with high specificity, requiring 100 times higher concentrations to inhibit other kinases. Preclinical studies, the results of which are described in Table 2, reported a 50% bioavailability of orally administered ZD1839 without drug-related toxicity despite EGFR being a widely expressed protein [24, 25]. Phase I studies of ZD1839 in solid tumors revealed peak plasma levels of drug 3-7 hours after a single oral dose, with a half-life ranging from 12-51 hours [24, 26]. Continuous, daily, oral administration with an intermittent schedule of 14 of 28 days revealed a longer median half-life of 46-49 hours [25, 27]. The dose-limiting toxicity was diarrhea with adverse effects including an acneiform rash, nausea, and vomiting. Phase I/II trials demonstrated partial responses to ZD1839 in non-small cell lung cancer and prostate cancer, with several patients claiming improved quality of life [25, 27, 28]. Phase I/II trials of single-agent oral ZD1839 have included breast cancer patients, but have not yet revealed any change in disease status [25, 27]. Furthermore, ZD1839 did not reduce tumor exposure to chemotherapy when administered as part of a combination regimen [25]. A phase II trial is currently being conducted by the Eastern Cooperative Oncology Group in which HER2-overexpressing, trastuzumab-naïve metastatic breast cancer patients are treated with combined ZD1839 and trastuzumab [29]. Blockade of the EGFR may prevent transactivation of HER2, improving response rates to the HER2 mAb trastuzumab. Such a combination may also be considered for trastuzumab-resistant tumors, which may no longer be responding to trastuzumab due to compensatory signaling by the EGFR.

OSI-774

OSI-774 (Tarceva™; formerly CP-358, 774; OSI Pharmaceuticals; Tarrytown, NY) is another orally active quinazoline that reversibly inhibits EGFR tyrosine kinase function. Preclinical studies reported an 80% bioavailability of orally administered OSI-774 with negligible drug-related toxicity at doses up to 15 mg/kg/day and emesis and gastrointestinal toxicity at higher doses. Phase I/II studies revealed that continuous administration of 150 mg/day of OSI-774 was well tolerated in patients with solid tumors including five breast cancer patients. The dose-limiting toxicity was diarrhea, occurring at 200 mg/day when administered on a continuous, daily schedule for 21 out of 28 consecutive days [30]. No dose-limiting toxicities were apparent when OSI-774 was given on a weekly schedule for 3-4 weeks at up to 1,600 mg/day. Adverse events included fatigue, headache, nausea, and an acneiform rash, which was similar to that produced by ZD1839 and occurred in 78% of patients [25]. Partial responses for renal and colon cancers and stable disease in prostate, non-small cell lung cancer, cervical, and head and neck cancers have been documented [30]. However, data regarding efficacy of OSI-774 against breast cancer have not yet been reported.

CI-1033

CI-1033 (PD183805; Pfizer; New York, NY) is another orally available quinazoline currently in clinical trials for solid tumors. In contrast to ZD1839 and OSI-774, CI-1033 irreversibly inhibits a region of the catalytic site conserved among all erbB receptors and is, therefore, called a pan-Her inhibitor [31]. CI-1033 is likely to be effective in a larger subset of breast cancer patients than EGFR-specific TKIs. Additionally, due to its irreversible binding to the kinase catalytic site, CI-1033 offers prolonged suppression of enzymatic activity, which may improve tumor response and require less frequent dosing.

Preclinical data are summarized in Table 2. Phase I trials have revealed an acceptable safety profile for CI-1033, with dose-limiting toxicities including diarrhea, acneiform rash, nausea, vomiting, and thrombocytopenia [22]. Pre- and post-treatment tumor biopsies were studied for biomarkers and showed 40%-50% reduced EGFR and HER2 phosphorylation, which correlated with decreased proliferation. Although partial remissions and stable disease occurred primarily in squamous cell skin cancer and advanced-stage non-small cell lung cancer, respectively, one heavily pre-treated breast cancer patient remained in a CI-1033 phase I trial for more than 6 months without disease progression [22]. Current clinical trials include testing CI-1033 in metastatic breast cancer patients who have failed trastuzumab therapy. As trastuzumab resistance is a considerable clinical problem that could potentially be due
to compensatory signaling by other Her receptors, pan-Her inhibitors like CI-1033 may offer a new therapeutic strategy to these breast cancer patients.

**HER2-TARGETED THERAPY**

The *neu* gene was discovered in tumors in nitrosourea-treated rats, where it was isolated as a dominant-acting oncogene with a mutation in the transmembrane region [32-35]. The human homolog, known as c-erbB2 or HER2 was identified in human tumors using hybridization with v-erbB and EGFR receptor probes and found to be amplified to 4-20 copies per cell [36, 37]. This gene is located at chromosome 17 q11-q12 and encodes a 185-kD transmembrane glycoprotein (p185HER2) having intracellular tyrosine kinase activity and an extracellular domain that is very similar to the EGFl-binding domain of the EGF receptor [38]. Amplification of the HER2 gene results in the overexpression of mRNA and protein having a normal sequence. No mutations have been identified in human breast cancer cells. Although the mechanism of gene amplification is not known, experimental data show that HER2 acts as a potent oncogene in vitro [39] and in vivo [40, 41]. The HER2 gene is amplified in approximately 20%-25% of invasive breast cancers (Table 1) [42, 43]. A correlation has been noted between HER2 gene amplification and/or protein overexpression and poor disease-free survival [42, 44-49]. HER2 overexpression has also been associated with resistance to chemotherapy [50, 51] and hormone therapy [52]. However, the clinical implications of those associations remain controversial [53].

The strong linkage to the pathogenesis of breast cancer and its association with prognosis made HER2 a target for the development of new cancer therapies [54, 55]. There have been a multiplicity of approaches, as outlined in Figure 1. The level of HER2 expression found in human cancer cells where gene amplification occurs is much higher than that found in normal adult tissues. A second attractive aspect of the HER2 target is that it is present in a very high proportion of tumor cells [56], and tumors with high expression (score 3+) show uniform intense immunohistochemical staining [45, 57]. This characteristic suggests that, in a given patient, anti-HER2 therapy should be able to attack nearly all cancer cells. Finally, the HER2 overexpression phenotype is apparently shared between the primary tumor and metastatic sites [58]. This is important in that it indicates that therapy for metastatic disease can be selected based on analysis of the primary tumor, and again indicates that an anti-HER2 therapy should be able to treat all sites of disease.

**Anti-HER2 Antibodies**

A number of studies have shown that mAbs directed against the HER2 protein can reduce the growth rate of human tumor cells [59-64] and sensitize them to chemotherapeutic agents [65, 66]. Although the mechanism of action of passive antibody therapy is not well defined, there is evidence that internalization may be necessary for biological activity [61, 65].

**Trastuzumab**

Trastuzumab (Herceptin®; Genentech; South San Francisco, CA) is the first HER2-directed therapy to gain approval from the U.S. Food and Drug Administration for the treatment of patients with metastatic breast cancer. Trastuzumab is a humanized mAb directed against the extracellular domain of the HER2 protein. Preclinical data on trastuzumab and its parental murine antibody, known as 4D5 or rhuMabHER2, are outlined in Table 2. Phase I trials of trastuzumab showed the antibody was safe, and pharmacokinetics were reliable. Response rates to trastuzumab given as a single agent ranged from 12%-40%, in part depending on the method used to determine HER2 status and the prior treatment received [67-69]. In a pivotal study, Slamon et al. [70] showed that combining trastuzumab with either doxorubicin plus cyclophosphamide (AC) or single-agent paclitaxel produced higher response rates and survival times than chemotherapy alone. However, the administration of AC plus trastuzumab caused severe cardiac dysfunction. This led to the development of trastuzumab-based combinations that do not contain anthracyclines [71, 72]. Regimens evaluated to date with promising results include cisplatin [73], weekly paclitaxel [74], docetaxel [75], vinorelbine [76], and gemcitabine [77]. Combinations of taxanes, platinum salts, and trastuzumab (TCH) are highly synergistic in vitro [78, 79]. Preliminary data from phase II studies of TCH have shown a promising high response rate and time to progression [80]. Slamon and colleagues [81] recently reported a time to progression of 17 months in patients with HER2-amplified metastatic breast cancer treated with docetaxel, carboplatin, and trastuzumab.

One of the lessons learned from the clinical development of trastuzumab is the importance of HER2 overexpression. It is now clear that only patients whose tumors carry HER2 gene amplification or high HER2 protein expression using immunohistochemistry (score 3+) benefit from trastuzumab-based therapy [69, 74, 75, 82]. Another method under investigation for predicting response to trastuzumab is the quantification of the extracellular domain (ECD) of the HER2 protein in the serum. Our group has recently shown that patients with high HER2 ECD levels at baseline had a higher response rate to docetaxel and trastuzumab therapy than patients who had low HER2 ECD levels prior to initiation of therapy [75]. A multicenter, prospective study is ongoing to evaluate the role of the HER2 ECD assay for patients with metastatic breast cancer undergoing trastuzumab-based therapy.
Perhaps the most promising application of trastuzumab mAb therapy will be in the adjuvant setting. Large randomized trials are being conducted by cooperative groups. The National Surgical Adjuvant Breast and Bowel Project (NSABP)-B31 protocol is randomizing node-positive, HER2+ breast cancer patients to either four cycles of AC followed by four cycles of paclitaxel or the same regimen plus trastuzumab (in combination with paclitaxel). The Breast Intergroup protocol N9831 is testing a similar sequential approach using weekly paclitaxel. In addition, trastuzumab is being administered either concomitantly with paclitaxel or after completion of AC and paclitaxel therapy [83]. Both studies allowed HER2 testing at local hospitals initially. However, a significant number of false positives were noted, and a more centralized testing approach was implemented to assure proper patient selection [83, 84]. The Breast Cancer International Research Group (BCIRG protocol 006) is evaluating the role of docetaxel with and without trastuzumab following AC chemotherapy. A third experimental arm incorporates the TCH regimen. This protocol includes node-negative and high-risk node-negative patients. HER2 status must be determined using fluorescence in situ hybridization at a central laboratory.

Novel Approaches to HER2-Antibody-Based Therapy

To increase the potency of antibody-directed therapy, the specificity of an antigen-binding site has been combined with a wide variety of effector agents. Toxins have been targeted to HER2 using chemical conjugation with intact antibodies [85]. Using this approach, trastuzumab has been linked to DM-1 toxin (preclinical studies ongoing). Another approach is to generate recombinant molecules in which an antibody combining site is fused directly to the toxin [85, 86]. These immunotoxins and oncotoxins are extremely potent molecules and show strong selectivity for HER2 binding. Recombinant toxins show promise in that they can be safely delivered to experimental animals at effective doses [87, 88]. Recombinant toxins are also attractive in that they are relatively small proteins and may be able to penetrate tumors more effectively. This is indicated by pharmacokinetic measurements showing that recombinant toxins readily leave the circulation [87, 89]. One potential limitation facing the development of toxin targeting is the potential for an immune response to the protein.

Radionuclides have also been attached to anti-HER2 antibodies. These have shown the ability to image tumors in experimental animals and in humans [90, 91]. The therapeutic application of radiolabelled antibodies to this and other types of cancer may depend on the attachment of isotopes of sufficient specific activity and suitable decay pathlength.

Approaches to immunotherapy have been developed that rely on targeting by anti-HER2 antibodies. Both are designed to deliver immune effector cells to the tumor. In the first approach, a single chimeric protein molecule is designed to have two antibody-binding specificities, one that binds HER2 and the other that binds an immune cell, either via CD16, Fc receptor III [92, 93], or CD3 [94]. Phase I clinical studies have allowed toxicity to be assessed, and there is evidence that a biologically relevant concentration of the experimental therapy can be achieved [95].

Inhibition of HER2 Synthesis

Since a primary event in inducing malignancy is HER2 gene amplification, several investigators have developed strategies to prevent the synthesis of mature HER2 at the cell membrane. The strategy most immediate to the underlying genetic defect derives from the finding that the HER2 gene can be repressed by the introduction of the adenovirus E1A gene [96]. Delivery of E1A expression constructs into human tumor cell lines using liposomes has resulted in inhibition of HER2 expression and loss of tumorigenicity [97]. A phase I clinical trial of E1A therapy showed that intracavitary injection of E1A gene complexed with DC-Chol cationic liposome (DCC-E1A) is feasible in patients with breast cancer [98]. Antisense approaches to limiting HER2 expression have also been reported [99, 100].

HER2 Vaccines

As described, the level of expression of HER2 seen in human tumors with gene amplification is unprecedented in normal tissues. Consequently, it has been proposed that peptides derived from the erbB2 sequence could be presented by the human major histocompatibility complex and recognized by the T-cell receptor on lymphocytes [101, 102]. Although the gene amplified in human tumors is apparently normal, tolerance might be broadened by a quantitative increase in epitope display. Consistent with this concept is the finding that T-helper, cytotoxic, and antibody responses have been identified in patients whose tumors overexpress HER2 [103]. As a consequence, several peptide sequences within erbB2 that mediate T-helper or cytotoxic responses have been identified [104, 105]. Such peptides, or potentially a larger expression construct containing more of the erbB2 coding sequence, might be used as a vaccine. No evidence of autoimmune consequences have been observed in these or in tumor-bearing patients [101].

STI571

STI571 (imatinib mesylate; Gleevec™; Novartis; Basel, Switzerland) was the first successful rationally developed receptor-targeted agent for chronic myelogenous leukemia.
This phenylaminopyrimidine derivative was selected from a screen of molecules for its ability to competitively target the ATP-binding site of the platelet-derived growth factor receptor (PDGFR) [106]. In vitro analysis revealed that STI571 also selectively inhibits the ABL and KIT (CD117) tyrosine kinase receptors. Initial phase I/II studies of STI571 were performed in CML patients who failed interferon therapy and in acute lymphoblastic leukemia (ALL) patients harboring constitutively active ABL due to bcr-abl chromosomal translocations (i.e., Philadelphia chromosome). CML patients in chronic phase (i.e., less than 15% blasts) demonstrated that the threshold maximum effective dose for STI571 is 300 mg, at which complete hematologic and cytogenetic responses were achieved in 98% and 13% of patients, respectively. Responses were still evident after a median follow-up time of almost 1 year [107, 108]. Kantarjian and colleagues [109] recently reported a phase II study of 532 chronic-phase CML patients previously treated with interferon alpha. In that study, 400 mg oral STI571 daily produced major cytogenetic responses in 60% of the 454 patients with confirmed chronic-phase CML and complete hematologic responses in 95%. Phase I studies in relapsed Bcr-abl+ ALL patients and CML patients in myeloid or lymphoid blast crisis demonstrated reduced marrow blasts of less than 5% in 21% of myeloid patients and 55% of lymphoid patients [107, 108]. Toxicity was low in all trials, although grade 2-3 myelosuppression was observed in 10%-20% of patients, and may represent the therapeutic effect of reduced lymphocyte counts.

STI571 has also demonstrated activity against conditions in which either KIT or PDGFR is activated. Activating mutations of c-kit (CD117) are common in gastrointestinal stromal tumors [110]. An open-label, randomized trial of 400 mg or 600 mg STI571 in 147 GIST patients demonstrated partial responses in 54% of patients and stable disease in 28%, but did not result in any complete responses [111]. Four patients with chronic myeloproliferative disease harboring an activating translocation of the PDGFR-β gene treated with STI571 demonstrated normal blood counts and reduced levels of PDGFR within 3 months of treatment initiation [112]. Additionally, a patient with unresectable metastatic dermatofibrosarcoma protubersans achieved 75% tumor shrinkage upon 4 months of STI571 treatment, allowing resection of the mass [113].

PDGFR and KIT are expressed in most breast tumors (Table 1). Autocrine stimulation of KIT and PDGFR by stem cell factor and PDGF, respectively, is observed in breast tumors and may enhance mitogenic signaling [108, 114]. Therapeutic inhibition of KIT and PDGFR by STI571 is being examined in a phase II study at the University of Texas M. D. Anderson Cancer Center in metastatic breast cancer patients.

### INSULIN-LIKE GROWTH FACTOR-I-RECEPTOR-TARGETED THERAPY

The insulin-like growth factor (IGF) mitogenic signaling pathway is an attractive therapeutic target in breast cancer as its ligands and receptors are frequently overexpressed and implicated in cellular proliferation, transformation, and metastasis [115]. The IGF system is composed of ligands IGF-I and IGF-II, receptors IGF-IR and IGF-2R, and at least six IGF binding proteins (IGFBPs). High circulating levels of IGF-I are associated with a greater risk of breast cancer in premenopausal women, with an especially high risk among those younger than 50 years [116]. Elevated expression of IGF-II is linked to poor prognosis in breast cancer [117]. The IGF-IR is highly expressed and activated in breast tumors, and loss of heterozygosity is observed at IGF-2R (also called the mannose-6-phosphate receptor) [118, 119]. Additionally, high levels of IGFs prevent apoptosis in response to chemotherapeutics and radiation, and overactive IGF-IR signaling is linked to resistance to trastuzumab in HER2-overexpressing breast cancer cells [120, 121].

The importance of IGF signaling in breast cells is highlighted by evidence of crosstalk with ER signaling, such that transcription of IGF-I, IGF-II, IGF-IR, and IGFBPs is activated by the ER, which itself activates expression of IGF-I [120]. Treatment of breast cancer patients with the antiestrogens tamoxifen or raloxifene decreased the IGF-I/IGFBP-3 molar ratio such that lower amounts of circulating IGF-I were available for mitogenic signaling [122, 123]. Somatostatin analogues also reduce IGF signaling by reducing IGF-I levels. RC-160 (vapreotide) significantly lowered IGF-I levels in heavily pretreated metastatic breast cancer patients but was unable to achieve objective tumor responses in phase II trials despite preclinical evidence of tumor inhibition [124]. Combination treatment with tamoxifen and the somatostatin analogue octreotide was also unable to achieve antitumor response [125]. However, a meta-analysis indicated that somatostatin analogues given as first-line therapy were associated with at least a partial tumor response with few side effects [126].

Several targeted therapies in preclinical development directly abrogate IGF signaling, including antisense strategies and antibody blockade. Antisense oligonucleotides against IGF-IR mRNA blocked proliferation of murine mammary carcinoma cells and demonstrated antitumor effects in vivo [127]. αIR3, a mouse anti-IGF-IR antibody, blocked IGF activity and tumor formation and growth in xenograft models of human breast cancer. However, αIR3 demonstrated some level of agonist activity as well as a cross-reaction with the insulin receptor. Furthermore, targeting IGF-IR alone may not be effective, since multiple
receptors mediate IGF signaling [115, 128-131]. Hence, targeting other components of the IGF pathway may be more beneficial.

IGFBPs bind and regulate circulating IGFs. Levels of IGFBP-1 and IGFBP-3 predicted recurrent disease at distant sites in breast cancer patients [132]. Subcutaneous delivery of a recombinant form of IGFBP-1 fused to polyethylene glycol (PEGBP-1) blocked IGF-I- and estrogen-mediated breast tumor growth in vivo [115]. Interestingly, IGFBP-3 restored sensitivity to trastuzumab in HER2+ breast cancer cells and, thus, may be a candidate for development to treat trastuzumab-resistant tumors [121].

**CONCLUSION**

The relevance of growth factor receptors and their signaling pathways to breast cancer is well established. Several clinical trials testing agents that directly interfere with these pathways are currently in progress. Treatments include mAbs that block receptor activation and tyrosine kinase inhibitors that competitively block ATP binding and prevent kinase signaling. Evidence to date suggests that direct targeting of growth factor receptors is a promising therapeutic strategy for breast cancers with abnormalities in these pathways. The challenge is to identify the patient population most likely to benefit from this biological therapy approach.

**ACKNOWLEDGMENTS**

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**Table 3. Growth-factor-receptor-targeting agents in clinical development**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Class of compound</th>
<th>Phase of development for metastatic breast cancer</th>
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<td>Imatinib mesylate (STI571)</td>
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<td>Novartis</td>
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<td>mAb-toxin conjugate</td>
<td>Preclinical</td>
<td>Genentech</td>
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<td>HER2</td>
<td>Transcriptional inhibitor</td>
<td>I</td>
<td>Targeted genetics</td>
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<td>HER2</td>
<td>mAb</td>
<td>I</td>
<td>Genentech</td>
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