Minireview

Targeting ErbB receptor signaling: A pan-ErbB approach to cancer

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Abstract

The ErbB receptors are localized to the cell membrane where they are activated by ligand to trigger a network of signaling pathways. In some cancer cells, dysregulation of ErbB-mediated signaling confers a growth advantage, resulting in cellular transformation and increased metastatic potential. Several agents that inhibit individual ErbB receptors have recently been approved for the treatment of human malignancies, validating ErbB receptors as therapeutic targets. One strategy to improve the efficacy of ErbB-targeted therapies is to inhibit multiple ErbB receptors, thereby interfering with the cooperation that exists between receptors. This minireview addresses the approaches being developed to concurrently inhibit multiple ErbB receptors. [Mol Cancer Ther 2004;3(10):1335–42]

Introduction

The ErbB receptors, epidermal growth factor receptor (EGFR; ErbB1), HER-2 (ErbB2), ErbB3, and ErbB4, function in concert to transmit signals across the cell membrane in a wide variety of epithelial cells. ErbB receptors share a common structural organization consisting of an extracellular ligand binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain (1–3). EGFR, ErbB3, and ErbB4 bind ligand to induce ErbB homodimerization or heterodimerization, whereas HER-2 has no identified ligand and functions as the preferred ErbB dimerization partner (1, 4, 5). ErbB dimerization results in the phosphorylation of intracellular tyrosine residues, activating diverse signaling pathways (1–3). These downstream pathways culminate in a variety of cellular responses, including cell growth, proliferation, differentiation, and migration (1, 2). Interactions between ErbB receptors allow HER-2, which has no identified ligand, and ErbB3, which is kinase dead, to participate in efficient signaling (1, 3, 4). In addition, interactions between ErbB

receptors provide a mechanism for signal diversification and amplification (1, 3). In this context, ErbB receptor complexes serve not only as signaling conduits but also as active determinants of cellular response.

Alterations in ErbB activation and expression have been recognized in several human malignancies. For example, increased expression of wild-type EGFR is commonly found in breast, lung, head and neck, bladder, and other cancers and is associated with a poor clinical outcome, albeit with some variability (2). Another example is provided by EGFR vIII, a constitutively activated form of EGFR harboring a rearrangement in the extracellular domain, found in glioblastoma (2). More recently, activating mutations in the tyrosine kinase domain of EGFR have been identified in a subgroup of patients with non-small cell lung cancer (6, 7). Perhaps the best example, however, is provided by amplification of the HER-2/*neu* gene, present in 25% to 30% of breast cancers, and associated with a statistically significant shortened disease-free and overall survival (8–10). In addition to these examples involving individual ErbB receptors, interactions between ErbB receptors have also been shown to play an important role in human malignancies. Preclinical experiments have shown that ErbB receptors act synergistically to transform NIH3T3 cells (11, 12), and some human cancers that overexpress both EGFR and HER-2 have a poorer prognosis than cancers that overexpress either receptor alone (13–15). Together, molecular alterations in ErbB receptors contribute to the malignant phenotype, providing justification for the development of ErbB-targeted therapies in the treatment of cancer.

The search for compounds that inhibit ErbB receptors has yielded agents with varying degrees of receptor specificity, including monoclonal antibodies, tyrosine kinase inhibitors, immunotoxin conjugates, antisense oligonucleotides, and bispecific antibodies. Among the classes of agents targeting ErbB receptors, the monoclonal antibodies and tyrosine kinase inhibitors, listed in Tables 1 and 2, respectively, are furthest in development. In general, monoclonal antibodies against the extracellular domain target an individual ErbB receptor (1), whereas tyrosine kinase inhibitors that compete with ATP binding may be more or less selective (16–24). The first ErbB-targeted compounds approved for use in human malignancies are trastuzumab (Herceptin), a monoclonal antibody directed against HER-2; cetuximab (Erbitux), a monoclonal antibody directed against EGFR; and gefitinib (Iressa), an EGFR tyrosine kinase inhibitor. Individually, these compounds target single ErbB receptors to generate tumor shrinkage. However, because members of the ErbB receptor family cooperate in signal transduction and malignant transformation,

Table 1. Selected ErbB-targeted monoclonal antibodies

Monoclonal Antibody	Type	Target	Stage of Development
Cetuximab (Erbitux, C225)	Human/mouse chimeric antibody	EGFR	Approved for colon cancer
Panitumumab (ABX-EGF)	Fully human antibody	EGFR	Phase II
Trastuzumab (Herceptin)	Humanized antibody	HER-2	Approved for HER-2-positive breast cancer
Pertuzumab (2C4)	Humanized antibody	HER-2	Phase II

there is increasing evidence to support the concurrent inhibition of two or more receptors. This minireview examines the scientific rationale, preclinical evidence, and early clinical results with compounds used alone and in combination to target multiple ErbB receptors.

ErbB Receptor Interactions

Studies targeting multiple ErbB receptors in the treatment of cancer are based on the fact that ErbB receptors preferentially function in concert to transmit signals. Although certain malignancies have been linked to the overexpression of individual receptors, efficient signal transduction relies on the coexpression of ErbB receptor family members. ErbB receptor interactions also provide a mechanism for the selection and regulation of cellular responses. From the binding of ligand to the activation of downstream pathways, ErbB receptors are interdependent.

EGFR, ErbB3, and ErbB4 accept cues from their environment by binding one of many potential ligands. These ligands, called epidermal growth factor (EGF)–related peptides, are divided into three functional groups: (*a*) EGF, transforming growth factor-α, and amphiregulin, which bind EGFR; (*b*) betacellulin, epiregulin, and heparin binding EGF-like growth factor, which bind EGFR and ErbB4; and (*c*) neuregulins 1 to 4 (NRG, also known as heregulins), which bind either ErbB4 alone (NRG-3 and NRG-4) or ErbB4 and ErbB3 (NRG-1 and NRG-2; refs. 3, 25). EGF-related peptides are synthesized as cell membrane–associated precursors that function in juxtacrine signaling by binding ErbB receptors on adjacent cells (3, 25).

Proteolytic cleavage releases soluble forms that function in autocrine and paracrine ErbB-mediated signaling (3, 25). Because most ErbB ligands act within close range, and different tissues show unique patterns of ligand expression, ligand availability is a mechanism of regulation for signal transduction (3).

On ligand binding, ErbB receptors partner either with like receptors to form homodimers or with other ErbB receptor family members to form heterodimers. Interactions between ErbB receptors are necessitated by inherent deficiencies in HER-2, which has no known ligand, and ErbB3, which is kinase dead (1). In isolation, neither HER-2 nor ErbB3 can initiate ligand-mediated signaling. Although wild-type *neu*, the rat homologue of HER-2, can spontaneously form active homodimers when present in high concentrations (26), it is unknown if ligand-independent signaling occurs in human malignancies that overexpress HER-2. Instead, it seems that HER-2 participates in the activation of downstream pathways through heterodimerization (1, 3, 4).

Recent crystallography studies have shown how ErbB receptors interact to form dimers (Fig. 1). ErbB receptor extracellular domains are composed of four subdomains: subdomains I and III mediate ligand binding, whereas cysteine-rich subdomains II and IV participate in receptor dimerization (27, 28). EGFR and ErbB3 receptors that are not bound by ligand demonstrate a closed configuration in which the dimerization loop from subdomain II is in contact with subdomain IV (29, 30). In this model, ligand binding effectively opens the receptor, releasing subdomain II from subdomain IV and exposing the dimerization

Table 2. Receptor specificities for selected ErbB tyrosine kinase inhibitors

Compound	Reversibility of Inhibition	Purified Enzyme Kinase Inhibition IC$_{50}$ (nmol/L)			Reference(s)
		EGFR	HER-2	ErbB4	
EGFR specific					
Gefitinib (Iressa, ZD1839)	Reversible	27-33	≥3,700	NR	(16)
Erlotinib (Tarceva, OSI-774)	Reversible	2	>1,000	NR	(17, 18)
EKB-569	Irreversible	83	1,229	NR	(19)
HER-2 specific					
CP-724,714	NR	4,300	8	NR	(20)
TAK165	NR	NR	NR	NR	(21)
Dual or pan-Erb					
Canertinib (CI-1033)	Irreversible	0.8	19	7	(22)
Lapatinib (GW-2016)	Reversible	11	9	367	(23)
PKI-166	Reversible	1	11	433	(24)

NOTE: NR refers to variables that were not reported in the preclinical reference.
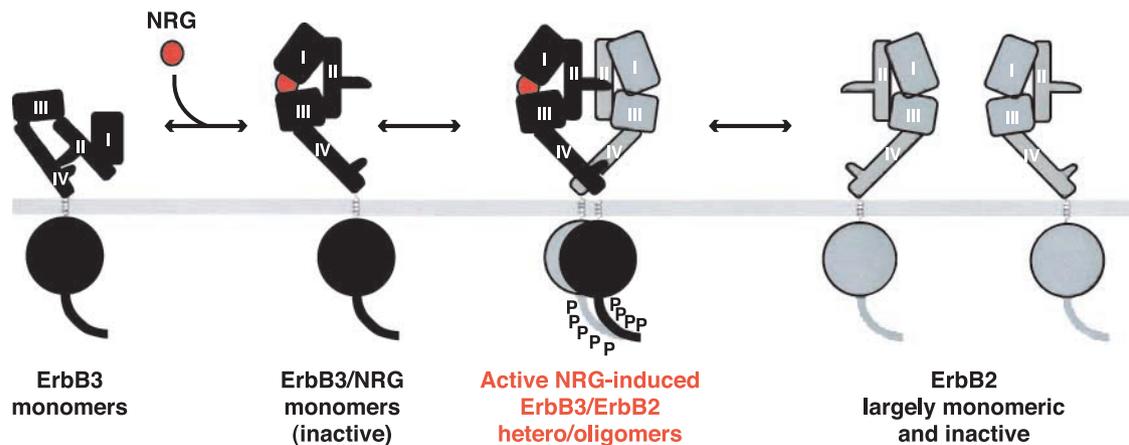
Figure 1. Model for ligand-induced heterodimerization of ErbB2 and ErbB3. An unbound ErbB3 monomer has a closed configuration (*left*). Ligand binding results in a change in configuration, exposing the dimerization loop. In contrast, a HER-2 monomer has a fixed open configuration (*right*). In the presence of ligand-bound ErbB3, HER-2 forms heterodimers with ErbB3. Reproduced from ref. 28 with permission from Elsevier.

loop to enable association with other receptors (31, 32). Results from recent structural studies disprove a previous model of ErbB dimerization in which ligand binding was thought to produce a ligand cross-link mediating dimerization (33). Instead, ligand binding at subdomains I and III is physically separate from the dimerization interface, and ErbB dimerization is mediated by a direct connection between two receptors (28).

In contrast to EGFR and ErbB3, which require ligand binding prior to dimerization, HER-2 is fixed in an open configuration in which the dimerization loop from subdomain II is constitutively exposed (34, 35). This configuration is a result of a unique interaction between subdomains I and III that mimics a ligand-activated state (28, 34, 35). Although the structure of HER-2 suggests that is poised to form both homodimers and heterodimers, experimental models have failed to detect significant levels of soluble HER-2 homodimers, perhaps because both the HER-2 dimerization loop and the HER-2 dimerization loop docking site are electronegative (28, 34, 35). In fact, the primary role of HER-2 is that of a coreceptor.

The process of dimerization positions two ErbB tyrosine kinases in close proximity, allowing transphosphorylation of the intracellular domain and initiation of a chain of downstream biochemical reactions. Although ErbB receptors share some common signaling pathways, individual ErbB receptors preferentially bind specific molecules based on the presence of defined tyrosine-containing motifs (3). For example, ErbB3 is the most efficient activator of phosphatidylinositol 3′-kinase because it has multiple repeats of the motif recognized by the p85 subunit (3). Importantly, the signals produced by an ErbB dimer are not simply determined by the signaling properties of its individual receptor components (3). Instead, ErbB receptors seem to acquire unique signaling properties through the process of transphosphorylation, which produces a dimer-specific pattern of phosphorylated tyrosine residues (3). This is illustrated by the difference between EGFR signaling in EGFR homodimers, which bind Shc, Cbl, and Grb2, and EGFR/ErbB4 heterodimers, which bind Shc but not Cbl or Grb2 (3, 36). In this manner, the composition of an ErbB dimer determines which downstream pathways are activated.

The amplitude and duration of the activated signals are also determined by the composition of the ligand-receptor complex. HER-2-containing heterodimers produce enhanced and prolonged activation of downstream pathways because HER-2 increases the affinity of its dimerization partner for ligand, decreases the rate of ligand-receptor complex internalization, and predisposes the receptor to recycling (1, 27). ErbB receptor trafficking has been studied in detail using EGFR homodimers and heterodimers. EGF-stimulated EGFR homodimers undergo rapid endocytosis and lysosomal degradation, whereas EGF-stimulated EGFR/HER-2 and EGFR/ErbB3 heterodimers remain at the cell surface for a longer period of time (37). This difference is due, at least in part, to the ability of EGFR homodimers to couple to Cbl, an E3 ubiquitin ligase that tags the receptor with ubiquitin, thereby sorting the ligand-receptor complex to internalization and eventual degradation (1, 27). Other ligand-activated ErbB receptor dimers bind Cbl less effectively and are endocytosis impaired (1, 27). Once internalized, these receptors are shunted to a default recycling pathway that presents individual ErbB receptors to the plasma membrane for repeat ligand activation (1, 27, 37).

The cooperation that exists between ErbB receptors may limit the success of agents that target individual receptors in the treatment of cancer. Preclinical studies have shown that cancer cells can be rescued from the antiproliferative activity of an agent directed against one ErbB receptor by the presence of ligand for another ErbB receptor. For example, the antiproliferative effect of the murine anti-HER-2 antibody 4D5 on HER-2-driven breast cancer cells is reversed by EGF-related peptides, and the ability of EGF-related peptides to stimulate HER-2-driven cancer cells is limited by a tyrosine kinase inhibitor with dual activity against EGFR and HER-2 (38). Using both *in vitro* and

in vivo models, strategies that employ a dual ErbB approach seem to have greater antitumor activity than agents targeting an individual ErbB receptor (39–44). In HER-2-positive breast cancer cell lines, trastuzumab shows additive and/or synergistic antitumor activity when combined with EGFR tyrosine kinase inhibitors (40–42), and in HER-2-positive BT-474 breast cancer xenografts, the combination of trastuzumab and erlotinib produces enhanced antitumor activity when compared with trastuzumab alone (39). Trastuzumab-resistant HER-2-positive breast cancer cells are sensitive to treatment with a combination of trastuzumab and an EGFR inhibitor (45) and to treatment with lapatinib, a single agent that inhibits both EGFR and HER-2 (46). A dual or pan-ErbB approach may have applications in malignancies other than HER-2-positive breast cancer. In MDA PCa 2a prostate cancer cells, the anti–androgen hydroxyflutamide is more efficacious when combined with both cetuximab and trastuzumab than when combined with either antibody alone (43). In OVCA 420 ovarian cancer cells, the combination of cetuximab and 4D5 also shows greater antiproliferative activity than either antibody alone (44). Taken together, these preclinical data indicate that the most efficient ErbB-targeted strategies may be those that inhibit multiple ErbB receptors in a dual or pan-ErbB approach. This may be achieved by (*a*) combinations of agents that target individual ErbB receptors, (*b*) single agents that target multiple ErbB receptors, and (*c*) agents that interfere with ErbB receptor interactions.

Combinations of Agents That Target Individual ErbB Receptors

With the increasing availability of agents that target individual ErbB receptors, the possible combinations incorporating these agents are numerous. This discussion will be limited to combinations employing trastuzumab and EGFR tyrosine kinase inhibitors in the treatment of HER-2-positive breast cancer. HER-2-positive metastatic breast cancer may be the best setting in which to test the concurrent inhibition of multiple ErbB receptors, because the pathogenesis and progression of the disease are driven by amplification of the HER-2/*neu* gene, and breast cancer patients can be selected for targeted therapy based on the presence of this molecular abnormality. Trastuzumab, the prototypical ErbB-targeted therapy, has a single agent response rate of 34% in women with metastatic HER-2/*neu*-positive breast cancer as measured by fluorescence *in situ* hybridization (47). Preclinical experiments have shown that trastuzumab has synergistic activity when combined with chemotherapeutic agents such as carboplatin, docetaxel, and vinorelbine (48), and clinical trials employing these combinations have reported impressive response rates (49, 50). In addition, the combination of trastuzumab with chemotherapy results in a statistically significant improvement in survival for women with HER-2-positive metastatic breast cancer when compared with chemotherapy alone (51). The current practice of combining trastuzumab and chemotherapy exposes patients to cytotoxic side effects, however, diminishing one of the potential benefits associated with targeted therapy. An alternative is to use trastuzumab in combination with other targeted therapies such as EGFR tyrosine kinase inhibitors.

EGFR tyrosine kinase inhibitors have shown single-agent activity in breast cancer cell lines that overexpress HER-2 and coexpress EGFR. The EGFR tyrosine kinase inhibitor gefitinib inhibits HER-2 phosphorylation in BT-474, SKBR-3, and MDA-361 human breast cancer cells at concentrations of ≥ 1 μmol/L (39, 40). These effects may be due to a direct inhibition of HER-2 or due to an inhibition of EGFR-mediated transphosphorylation of HER-2. The selectivity of small-molecule inhibitors is concentration dependent, and gefitinib inhibits purified EGFR and HER-2 tyrosine kinases at IC_{50} values of 0.033 and ≥ 3.7 μmol/L, respectively (shown in Table 2; ref. 16). Thus, at the concentrations employed in the breast cancer cell line studies, it is unclear whether the anticancer activity of gefitinib is due to direct or indirect effects on HER-2. Regardless of the mechanism, these results suggest a potential role for EGFR tyrosine kinase inhibitors in the treatment of HER-2-positive breast cancer.

Preclinical studies have shown enhanced activity when trastuzumab is combined with EGFR tyrosine kinase inhibitors in HER-2-positive breast cancer models. In six of seven human breast cancer cell lines (SKBR-3, BT-474, MDA-361, UACC 812, SUM 190, and SUM 225) with increased levels of HER-2 and variable levels of EGFR expression, trastuzumab and the EGFR tyrosine kinase inhibitor erlotinib showed synergistic activity across a range of clinically relevant concentrations (42). The remaining cell line, MDA-453, was not sufficiently sensitive to erlotinib to allow for analysis by the median effects method (42). Similar results were obtained when trastuzumab was combined with gefitinib in the treatment of BT-474 and SKBR-3 cell lines, demonstrating either additive (40) or synergistic (41) antitumor activity. *In vivo*, trastuzumab and gefitinib produce enhanced antitumor activity in BT-474 xenografts (39). Moreover, trastuzumab-resistant BT-474 xenografts are sensitive to either erlotinib alone or in combination with trastuzumab without any obvious toxicity (45), suggesting that trastuzumab resistance may be overcome by the addition of EGFR-specific inhibitors.

These preclinical results serve as the rationale for a clinical trial of trastuzumab and erlotinib in patients with metastatic HER-2-positive breast cancer currently being conducted at University of California at Los Angeles (Los Angeles, CA). This trial is open to women with metastatic breast cancer with amplification of the HER-2/*neu* gene as measured by fluorescence *in situ* hybridization. Although tumor blocks must be available for measurement of EGFR expression, demonstration of EGFR expression is not required for participation. In addition to general inclusion and exclusion criteria such as defined adequate renal, hepatic, and hematologic function, patients must have a normal cardiac ejection fraction and a cumulative anthracycline exposure of ≤ 450 mg/m^2. The phase I portion of the trial was done to determine the recommended dose of continuous daily p.o. erlotinib administered in

combination with standard-dose weekly i.v. trastuzumab. Sixteen patients, 14 of whom were evaluable for response, received standard-dose trastuzumab in combination with escalating doses of erlotinib administered at the following dose levels: 50, 100, and 150 mg. The most common toxicities were grade 1 and 2 diarrhea and grade 1 and 2 rash (52). Two previously untreated patients enrolled at 150 mg developed confirmed partial responses, and one heavily pretreated patient, initially treated at 50 mg and subsequently escalated to 100 mg, was on study for >1.5 years with stable disease (52). Overall, this regimen is well tolerated and shows preliminary evidence of activity at the recommended dose of 150 mg erlotinib administered in combination with standard-dose weekly trastuzumab. The phase II portion of this trial is currently open to accrual for patients who have received no prior cytotoxic chemotherapy and/or trastuzumab in the treatment of metastatic HER-2-positive disease.

A parallel clinical trial of weekly i.v. trastuzumab and continuous daily p.o. gefitinib was performed through the Eastern Cooperative Oncology Group. This trial requires metastatic breast cancer that shows either HER-2/*neu* gene amplification by fluorescence *in situ* hybridization or HER-2 protein overexpression that is 3+ by immunohistochemistry. The phase I portion reported dose-limiting diarrhea when 500 mg gefitinib was combined with standard-dose weekly trastuzumab (53). The phase II portion enrolled patients at the recommended dose of 250 mg gefitinib administered in combination with weekly trastuzumab (53). Trials examining trastuzumab in combination with either erlotinib or gefitinib will be instrumental in defining the role for a dual ErbB approach in metastatic HER-2-positive breast cancer.

As an ErbB-targeted approach, the combination of a monoclonal antibody and a small-molecule tyrosine kinase inhibitor uses two agents with different sites of action and unique pharmacologic properties, providing a theoretical advantage over a single agent that either targets multiple ErbB receptors or interferes with ErbB interactions. The potential mechanisms of action of trastuzumab combined with an ErbB tyrosine kinase inhibitor include receptor down-regulation, signaling perturbation, angiogenesis inhibition, and antibody-dependent cell-mediated cytotoxicity (2, 54, 55). A monoclonal antibody is a large molecule with poor tumor and central nervous system penetration (56, 57), an obstacle that may be overcome by the concurrent administration of a tyrosine kinase inhibitor. However, because monoclonal antibodies require i.v. administration, combinations employing monoclonal antibodies and tyrosine kinase inhibitors may become cumbersome, especially if similar antitumor activity can be achieved by the administration of a single p.o. agent.

Single Agents That Target Multiple ErbB Receptors

The single agents that target multiple ErbB receptors are the dual or pan-ErbB tyrosine kinase inhibitors. Most tyrosine kinase inhibitors compete with the ATP binding site to inhibit phosphorylation. Because this site is highly conserved among ErbB receptors and, for that matter, among other kinases, tyrosine kinase inhibitors aimed at a particular ErbB receptor lose specificity at high concentrations. This is shown in Table 2, which lists the IC_{50} values for inhibition of ErbB tyrosine kinases in purified enzyme reactions with several tyrosine kinase inhibitors. Initial discovery processes sought to identify agents that were specific, thereby reducing potential toxicities. However, the body of preclinical evidence supporting the concurrent inhibition of multiple ErbB receptors has led to the development of dual or pan-ErbB tyrosine kinase inhibitors. Among these agents, canertinib, targeting EGFR, HER-2, and ErbB-4, and lapatinib, targeting EGFR and HER-2, are the furthest in development.

Canertinib (CI-1033) is an irreversible pan-ErbB inhibitor that produces prolonged inhibition of ErbB-mediated signaling (22). In purified enzyme reactions, canertinib has an IC_{50} value of 0.8 nmol/L for EGFR, 19 nmol/L for HER-2, and 7 nmol/L for ErbB4, demonstrating activity against all catalytically active members of the ErbB family (22). In a phase I clinical trial employing weekly administration schedule, canertinib was associated with thrombocytopenia and rare hypersensitivity reactions at doses >500 mg (58). This led to additional phase I trials exploring the feasibility of more frequent administration schedules. In these trials, canertinib was well tolerated and the principal dose-limiting toxicities were diarrhea, rash, and stomatitis, depending on the schedule employed (22). Phase I trials have shown evidence of target modulation following treatment with canertinib, with decreased levels of phospho-Erk and phospho-Akt (22). Phase II clinical trials are currently under way.

Lapatinib (GW2016) is a quinazoline derivative that functions as a reversible, dual ErbB tyrosine kinase inhibitor. In purified enzyme reactions, lapatinib has an IC_{50} value of 11 nmol/L for EGFR and 9 nmol/L for HER-2, demonstrating >300-fold selectivity compared with the other kinases tested (23). In a panel of tumor cell lines that overexpress either EGFR or HER-2, the IC_{50} values for growth inhibition are <0.16 μmol/L (23). Both *in vitro* and *in vivo*, lapatinib inhibits EGFR and HER-2 phosphorylation, resulting in decreased activation of downstream effectors Erk-1/2 and Akt (59). Unlike trastuzumab, lapatinib inhibits phosphorylation of p95[HER-2], a fragment of the HER-2 receptor produced by proteolytic cleavage of the extracellular domain (60), and lapatinib has been shown to inhibit the growth of trastuzumab-conditioned HER-2-positive breast cancer cells at concentrations similar to those required for trastuzumab-naive cells (46). In a phase I clinical trial of continuous daily p.o. lapatinib, grade 3 diarrhea was observed with twice-daily dosing, but doses of up to 1,800 mg/d were well tolerated (61). The most common toxicities observed with once-daily dosing were grade 1 to 2 rash, diarrhea, nausea, vomiting, constipation, anorexia, and fatigue (61). Importantly, lapatinib has shown clinical activity in malignancies refractory to agents that target individual ErbB receptors. In a phase I clinical trial of single agent continuous p.o. lapatinib, two patients

with non-small cell lung cancer resistant to gefinitib experienced minor responses (61), and in a phase II clinical trial in patients with trastuzumab-refractory metastatic breast cancer, 2 of the 41 patients evaluable at the time of a planned interim analysis experienced partial responses (62). Lapatinib is currently being explored alone and in combination with trastuzumab in the treatment of patients with metastatic HER-2-positive breast cancer. In addition, randomized trials combining lapatinib with either letrozole or capecitabine are ongoing.

As an ErbB-targeted approach, tyrosine kinase inhibitors are convenient because they may inhibit multiple ErbB receptors with a single p.o. agent. Like ErbB-targeted antibodies, tyrosine kinase inhibitors promote receptor down-regulation, interrupt signaling, and inhibit angiogenesis (2, 55). ErbB tyrosine kinase inhibitors also inhibit truncated ErbB receptors, a potential advantage over monoclonal antibodies if, in fact, truncated receptors play a significant role in signaling (60). As small molecules, tyrosine kinase inhibitors are able to penetrate the blood-brain barrier (63), indicating that these agents may be therapeutic in patients with primary or metastatic central nervous system disease. However, tyrosine kinase inhibitors are metabolized by the $P450$ enzyme system (64) and have a greater potential for drug interactions than antibodies.

Agents That Interfere with ErbB Receptor Interactions

Another approach to inhibiting multiple ErbB receptors is provided by pertuzumab (Omnitarg, 2C4), a monoclonal antibody against HER-2 that interferes with ErbB receptor interactions. Pertuzumab binds to a different epitope of the HER-2 extracellular domain than trastuzumab (34, 65) and seems to differ from trastuzumab in its mechanism of action. Trastuzumab is only active in cells that overexpress HER-2, and it does not directly affect the ability of HER-2 to function as a coreceptor (66, 67). In contrast, pertuzumab is active in cells that do not overexpress HER-2, and it inhibits ligand-mediated signaling by preventing the recruitment of HER-2 into ligand/ErbB receptor complexes (67, 68). In GEO colon cancer cells, pertuzumab blocks heregulin-stimulated phosphorylation of HER-2 and ErbB3, thereby inhibiting activation of mitogen-activated protein kinase and phosphatidylinositol 3′-kinase/Akt, resulting in decreased anchorage-independent growth (69). In the GEO model, pertuzumab also blocks EGF-stimulated phosphorylation of HER-2, inhibiting phosphatidylinositol 3′-kinase/Akt and anchorage-independent growth, but pertuzumab does not affect EGF-stimulated phosphorylation of EGFR nor does it inhibit EGF-stimulated activation of mitogen-activated protein kinase (69). In this manner, pertuzumab provides a unique opportunity to study the contribution of individual heterodimers to the activation of specific signaling pathways. Moreover, pertuzumab has shown clinical activity in malignancies that are not HER-2 driven, with responses reported in ovarian cancer, prostate cancer, and islet cell cancer (70). I.v. pertuzumab is well tol-

erated at doses up to 15 mg/kg every 3 weeks (70), and pertuzumab is currently being studied in phase II trials in hormone-refractory prostate cancer and advanced refractory or recurrent ovarian cancer. Future clinical directions are likely to include studies combining pertuzumab and trastuzumab, because this combination has synergistic anti-tumor activity in HER-2-positive breast cancer cells (71).

Future Directions

Advances in molecular biology have shown the importance of ErbB interactions in solid tumors, providing the rationale for therapeutic approaches that target multiple ErbB receptors. Several regimens are currently being tested in the clinic, including (*a*) combinations of agents that target individual receptors, (*b*) single agents that target multiple ErbB receptors, and (*c*) agents that interfere with ErbB receptor interactions. Differences between these approaches are blurring, as current trends include combining dual or pan-ErbB tyrosine kinase inhibitors with monoclonal antibodies and combining different classes of monoclonal antibodies. With the increasing availability of agents for clinical evaluation, candidate drugs and drug combinations must be selected based on robust preclinical data.

There is preliminary evidence to suggest that dual or pan-ErbB therapeutic approaches may have clinical activity where agents that target individual ErbB receptors have failed. Lapatinib has produced minor responses in the treatment of patients with gefitinib-resistant non-small cell lung cancer (61) and partial responses in the treatment of patients with trastuzumab-resistant HER-2-positive breast cancer (62). In addition, the combination of trastuzumab and erlotinib has produced disease stabilization for >18 months in a patient with HER-2-positive breast cancer who had previously received trastuzumab and chemotherapy (52). This antitumor activity has been achieved with relatively little toxicity, and the inhibition of multiple ErbB receptors does not seem to increase the dermatologic and gastrointestinal effects observed with EGFR inhibitors or the cardiac toxicity observed with trastuzumab. However, a direct comparison in a randomized clinical trial is required to determine whether a pan-ErbB approach provides improved clinical efficacy compared with an agent that targets an individual receptor.

The success of ErbB-targeted therapies in the clinic depends on the identification of appropriate patient populations. Because breast cancer patients can be selected for HER-2-targeted therapy based on the presence of HER-2/*neu* gene amplification, some of the initial studies employing a dual or pan-ErbB approach are being done in this disease. As molecular predictors of antitumor activity for ErbB-targeted therapies are defined in other malignancies, therapeutic strategies that inhibit multiple ErbB receptors may be applied. Recently, mutations in the region encoding the ATP binding pocket of the EGFR tyrosine kinase domain were identified in non-small cell lung cancer patients with durable clinical responses to either gefitinib or erlotinib (6, 7, 72). Although this suggests that lung cancer patients may soon be selected for treatment with

EGFR tyrosine kinase inhibitors based on the presence of these activating mutations, some caution is required. The apparent low mutation rate does not seem to explain the fact that erlotinib produces a 9% improvement in survival at 1 year in patients with non-small cell lung cancer (73, 74); thus far, mutations have not been detected in non-small cell lung cancer patients who achieved stable disease (72). Nevertheless, the discovery of EGFR mutations has potential applications for dual or pan-ErbB inhibitors. For example, lapatinib targets the ATP binding site, and mutations encoding changes at this site may affect its antitumor activity. Furthermore, because mutations involving the ATP binding site are thought to be activating, patients with malignancies harboring these mutations may benefit from more complete inhibition of ErbB signaling through dual or pan-ErbB therapeutic approaches. The search is under way for EGFR mutations in malignancies other than non-small cell lung cancer and for similar mutations involving other ErbB receptors. Thus, the manner in which patients are selected for ErbB targeted agents is currently in a state of flux.

A natural extension to the pan-ErbB approach is the incorporation of inhibitors of ErbB effector molecules. In this way, both horizontal and vertical ErbB receptor interactions may be targeted. Several agents that interfere with pathways downstream from ErbB receptors are currently available for evaluation, including farnesyl transferase inhibitors, mammalian target of rapamycin inhibitors, and angiogenesis inhibitors. A phase I/II clinical trial of trastuzumab and bevacizumab (a monoclonal antibody targeting vascular endothelial growth factor) is currently under way at University of California at Los Angeles in HER-2-positive breast cancer. If this strategy proves successful, approaches that achieve more complete inhibition of ErbB receptors may be evaluated in combination with inhibitors of downstream pathways. The ultimate objective is to replace the current cytotoxic-containing regimens and their attendant morbidity with potentially more effective and less toxic biologically targeted combinations.

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