THE erbB FAMILY: Targets for Therapeutic Development Against Cancer and Therapeutic Strategies Using Monoclonal Antibodies and Tyrosine Kinase Inhibitors

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Abstract The overexpression and aberrant function of members of the erbB family of receptors, particularly erbB1 (also known as epidermal growth factor receptor), and its ligands in many human cancers have provided a rationale for targeting this signaling network with novel approaches. erbB1 is a selective target for inhibiting cancers because its activation often confers a proliferative advantage. Activation of the erbB1 tyrosine kinase provides signals that drive dysregulated proliferation, invasion, metastasis, angiogenesis, and cell survival, and its inhibition has potential in both the treatment and prevention of these malignancies. Based on the structure and function of erbB1, two therapeutic strategies have been developed. The first uses human monoclonal antibodies (MAbs) generated against the receptor’s ligand-binding extracellular domain. These MAbs block binding of receptor-activating ligands, and, in some cases, can induce receptor endocytosis and downregulation. The second uses small molecules that compete with adenosine triphosphate (ATP) for binding to the receptor’s kinase pocket, thereby blocking receptor activation and the transduction of postreceptor signals. Early clinical studies suggest that both approaches are well tolerated and can induce clinical activity in many common malignancies.

INTRODUCTION

Cells are continuously exposed to diverse external stimuli, ranging from soluble endocrine and paracrine factors to signaling molecules on neighboring cells. The cell must interpret these extracellular signals to produce an appropriate developmental or proliferative response. Receptors of the tyrosine kinase (TK) family play principal roles in these processes, as they integrate a multitude of external
stimuli with specific internal signals and responses, ultimately allowing the cell to respond correctly to its environment. This review focuses on one family of structurally related TK receptors, known as the erbB type 1 receptors [also known as the epidermal growth factor receptor (EGFR) family], which are critical for mediating the proliferation and differentiation of normal cells. Experimental data support the suggestion that aberrant activation of the kinase activity of these receptors is important in the development and/or progression of human cancer. This article reviews current knowledge of the erbB family and its ligands, particularly their roles in signal transduction and the malignant phenotype, providing support for erbB, particularly erbB1, as a critical target for therapeutic development against malignant diseases.

**Evolution and Signaling Diversity of the erbB Family**

The erbBs were first implicated in cancer in the early 1980s, when the avian erythroblastosis tumor virus was found to encode an aberrant form of the human EGFR, or erbB1. Over the past several decades, four members of the erbB receptor family have been identified, and the physiologic function of these receptors and their ligands, the mediation of cell-to-cell interactions, and the consequences of erbB dysregulation are being appreciated, including their associations with the malignant process.

**erbB Family Ligands**

Most ligands of erbB family receptors are synthesized as transmembrane precursors that can be proteolytically cleaved to release the soluble form of the peptide or can function as membrane-anchored ligands in juxtacrine signaling (1–5). The peptides share a domain of homology that encompasses ~50 amino acids. The salient feature of this domain is the EGF-like or EGF-homologous region, which is required for erbB binding and activation. Expression and processing of ligand precursors are highly regulated, in part by extensive horizontal connections with other modulators and signaling systems. erbB ligands have been classified into three major groups based on their direct binding to a particular erbB family member (Figure 1). The first group consists of epidermal growth factor (EGF), transforming growth factor–alpha (TGF-α), and amphiregulin (also known as schwannoma-derived growth factor), which bind exclusively to erbB1. The membrane-bound forms of EGF and TGF-α may interact with receptors on the surface of adjacent cells, thereby potentially contributing to cell-to-cell adhesions and cell-to-cell stimulatory interactions. The second group of erbB ligands is represented by heparin-binding EGF, betacellulin, and epiregulin. Heparin-binding EGF and betacellulin bind and activate both erbB1 and erbB4, whereas epiregulin appears to be a more broad-spectrum erbB receptor ligand, binding all receptors except homodimers of erbB2 (6, 7). The third group of erbB ligands consists of a large and complex family of polypeptides called heregulins or neuregulins (NRGs) (also known as neu differentiation factors). Different NRG isoforms have varying
Figure 1  Structure of erbB family receptors and their cognate ligands. The receptor consists of three domains: a ligand-binding extracellular domain containing two cysteine-rich regions (CR1 and CR2), a transmembrane domain, and an intracellular domain containing a tyrosine kinase region. (EGF, epidermal growth factor; EGFR, EGF receptor; HER, human epidermal receptor; HB-EGF, heparin-binding EGF; NRG, neuregulin; TGF-α, transforming growth factor-α)

affinities for different receptor heterodimers, resulting in distinct but overlapping patterns of biologic responses (5).

Ligand-Receptor Signaling Specificity

Because many erbB ligands bind and activate the same receptor, functional redundancy, as well as specificity, is evident. TGF-α and EGF are almost indistinguishable in their ability to bind, activate, and downmodulate erbB1, but their biologic activities differ substantially. TGF-α is more potent than EGF as an angiogenic factor in vivo and in stimulating epidermal cell-colony formation in tissue culture (4, 5). Ligands such as EGF and NRG-4, which bind to erbB1 and erbB4, respectively, have narrow specificities, whereas others, such as epiregulin NRG-1β and betacellulin, bind to two distinct primary receptors. Overexpression of erbB2, which favors receptor heterodimer formation, can broaden ligand specificity, and ligands that are more efficient at recruiting this coreceptor can reduce the binding of less effective ligands. Splice variants of NRGs and various ligand-receptor complexes also differ in their ability to recruit any particular partner receptor, which affects their potency and the kinetics of signaling.

erbB RECEPTOR: STRUCTURE AND FUNCTION

All erbB receptor proteins belong to subclass I of the superfamily of receptor TKs (RTKs), classified according to their sequence homology and domain organization. erbB receptors are expressed in a variety of tissues of epithelial,
mesenchymal, and neuronal origin, where they play fundamental roles in critical developmental, proliferative, and differentiation processes (5–11). The erbB family consists of four closely related transmembrane receptors: erbB1 (also termed EGFR or HER1), erbB2 (also termed HER2 or Neu), erbB3 (also termed HER3), and erbB4 (also termed HER4).

With few exceptions (e.g., hematopoietic cells), erbB receptors are expressed in cells of mesodermal and ectodermal origins. In epithelial tissues, the basolateral distribution of erbB family members enables them to mediate signals required for growth between mesenchymal and epithelial tissue components. All four erbB receptors share a common molecular architecture composed of three distinct regions: (a) an extracellular region consisting of four glycosylated domains, two of which are cysteine-rich; (b) a transmembrane domain containing a single hydrophobic anchor sequence; and (c) an intracellular region containing the catalytic TK domain, which is responsible for the generation and regulation of intracellular signaling (Figure 1) (5–11). The formation of erbB homodimers and heterodimers, following ligand binding and receptor aggregation, activates the intrinsic RTK activity via intramolecular phosphorylation and generates a cascade of downstream chemical reactions that transmit a wide variety of cellular effects (Figure 2).

**erbB1**

erbB1 is essential to the regulation of normal cell growth and differentiation, and its dysregulation confers a proliferative advantage and malignant potential. The receptor transmits growth regulatory signals, particularly upon binding of EGF or TGF-α. In fact, except for the NRGs, all EGF ligands are capable of binding to the erbB1 receptor and producing mitogenic effects on EGF-responsive cells. erbB1 expression, overexpression, or dysregulation may alter intracellular

![Figure 2](https://example.com/figure2.png)  
*Figure 2*  Cellular responses associated with signaling through erbB family receptors.
signaling along pathways such as the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) signaling pathways. When activated, these pathways translate proteins required for G1 to S phase traverse or phosphorylation of antiapoptotic proteins leading to cell survival, respectively (Figure 2) (5–11). erbB1 currently serves as a target for therapeutic development against malignant diseases due to its ubiquitous nature and principal function as a regulator of proliferative signals; however, it is important to consider that most malignant tumors also have altered expression of erbB family members.

**erbB2**

The second member of the erbB receptor family to be discovered was erbB2 (HER2) which shows considerable homology to erbB1. Since erbB2 is a more potent oncoprotein than other members of the erbB family and has no known high-affinity ligands, its function is somewhat uncertain (7, 12). The discovery that erbB1 and erbB2 could form heterodimeric complexes raised the possibility that the interaction of ligands with erbB2 involved heterodimerization of erbB2 with other erbB receptors. Subsequent studies showed that erbB3 and erbB4 can also form heterodimers with erbB2. In the absence of a high-affinity ligand that directly binds to erbB2, it is likely that heterodimerization and transmodulation of other erbB receptors is the preferred initiating event for signaling (7, 9–11). There is increasing evidence that the principal function of erbB2 is as a coreceptor or dimerization partner for all other erbB family members and that it is important in the potentiation of erbB signaling.

**erbB3 and erbB4**

erbB3 (HER3) and erbB4 (HER4) are structurally related family members, although relatively little is known about their function (5–11). Interestingly, erbB3 lacks TK activity and is activated by TKs on other receptors. Heterodimers formed with erbB3 and erbB4 preferentially signal through the PI3K survival pathway relative to other types of heterodimers.

**SIGNALING THROUGH erbB**

On ligand binding, cellular responses are elicited through multiple divergent pathways (5–11). Briefly, ligand binding leads to receptor aggregation, facilitating the formation of both erbB homodimers and heterodimers, which are capable, to varying degrees, of activating the intrinsic receptor TK activity via intermolecular phosphorylation within its cytoplasmic domain. The resultant phosphorylated tyrosine residues serve as, or modulate the readiness of, docking sites for downstream signaling molecules and cytoplasmic messenger proteins, which, in turn, initiate a cascade of signals that emanate from the cytoplasm to the nucleus. Key tyrosine phosphorylation sites responsible for recruitment of downstream receptor targets are located in the juxtamembrane region and C-terminal tail of the receptor,
which flank the TK domain. Signaling through erbB1 and other family members triggers a rich network of downstream cellular pathways, culminating in responses that range from cell division to cell death, motility to adhesion, invasiveness, and angiogenesis. Ultimately, downstream effects on gene expression determine the biologic response to receptor activation. Because the network is often dysregulated in cancers, a molecular understanding of these processes may lead to the development of therapeutics.

**Signaling Diversity**

The ability of the erbB family to undergo homodimerization and heterodimerization, both constitutively and in response to their different ligands, provides enormous diversity to erbB-related signaling. Receptors that do not bind a particular ligand when expressed alone can be cross-activated if a binding-competent receptor is also present. For example, although EGF does not bind to or activate erbB2 by itself, EGF induces phosphorylation of tyrosine residues on both erbB1 and erbB2 in cells expressing both receptors (7, 9). The diversity and hierarchy of heterodimeric receptor interactions may be due to differences in the affinities of the various ligand-receptor-receptor complexes. Although 10 possible dimeric complexes can potentially form, each having variable potential to induce downstream signaling, there appears to be a graded ligand-dependent hierarchy for the formation of heterodimers, and those containing erbB2 are the most stable and preferred (7, 13, 14). erbB2, which does not have a direct high-affinity ligand, acts instead as a common receptor for other erbB family members. When erbB2 is overexpressed, heterodimers form preferentially. erbB2-containing heterodimers have features (e.g., slow ligand dissociation, relaxed ligand specificity, slow endocytosis, rapid recycling, prolonged firing) that prolong and enhance downstream signaling and its effects such as proliferation, migration, and resistance to apoptosis (7). The interactions of erbB family members are also characterized by directionality. For example, NRG-1 induces formation of erbB1-erbB3 heterodimers and erbB1-erbB4 heterodimers more readily than EGF does (7).

The specificity, potency, and diversity of intracellular signals are determined, in part, by positive and negative effectors of erbB proteins, the identity of the ligand, dimer composition, and specific structural determinants of the receptors. However, the principal determinant is the vast array of phosphotyrosine-binding proteins that associate with structurally diverse C-terminal “downstream docking” tails of each erbB receptor after engagement into dimeric complexes. These critical sequences, which contain tyrosine residues that undergo phosphorylation on ligand binding and receptor dimerization, represent docking sites for various proteins involved in signal transduction (7, 10, 13–15). Docking sites are provided for proteins containing Src homology 2 or phosphotyrosine-binding domains, which recognize specific phosphotyrosine residues in the context of their surrounding amino acids. Each erbB receptor displays a distinct pattern of C-terminal autophosphorylation sites. At least for erbB2, which does not have a direct activating ligand, these
phosphotyrosine-binding sites are essential for its transforming properties. There is a great deal of overlap among the signaling pathways activated by the four erbB receptors. For example, the MAPK pathway is an invariable target of all erbB family members. On the other hand, there are also specific examples of preferential modulation of specific pathways. This is illustrated by the presence of multiple binding sites for the regulatory subunit of PI3K on erbB3 and erbB4, which render these receptors the most efficient activators of the PI3K pathway (16). Simultaneous activation of cascades, such as the MAPK pathway, the stress-activated protein kinase cascade, protein kinase C, and the PI3K pathway, translates in the nucleus into distinct transcriptional programs, the culmination of which is the net cellular response.

**Turning Off the Signal**

The principal process by which erbB signaling is turned off is ligand-mediated receptor endocytosis, and the kinetics of this process is often understated with regard to the overall magnitude of signaling (7, 17, 18). The kinetics of signal degradation are determined in part by the composition of the receptors. For erbB, ligand stimulation results in rapid endocytosis and degradation of both the receptor and ligand. Ligand binding induces receptor clustering in clathrin-coated pits on the cell surface, followed by endocytosis, migration to multivesicular bodies, and eventual lysosomal degradation. Degradation of erbB depends on TK activity, and kinase-negative receptor mutants generally recycle to the cell surface for reutilization. erbB1 is more prone to degradation via endosome formation and hydrolysis and is the only erbB receptor that can interact directly with c-Cbl, a ubiquitin ligase that targets the erbB to lysosomal degradation following ligand-induced receptor internalization, whereas the other erbB receptors are relatively endocytosis impaired and tend to be recycled back to the cell surface (7, 17–19). The rapid endocytosis and degradation of the activated erbB receptor attenuate the signal generated at the cell surface in response to growth-factor stimulation. The particular mode and site of degradation are also determined in part by the composition of the dimer. For example, erbB1 homodimers are processed primarily to the lysosome, erbB3 molecules are constitutively recycled, and heterodimerization with erbB2 decreases the rate of endocytosis and increases recycling of its partners (7, 17–19). erbB2 homodimers, which are stable in the endosomal vacuole, are rapidly tagged with ubiquitin and processed for digestion, resulting in weak signals, whereas erbB2 heterodimers are relatively unstable in the endosome, resulting in a lower rate of degradation and a higher rate of receptor recirculation (7, 17–21).

To make matters even more complex, networks integrate heterologous signals from other networks. In the case of erbB, heterologous signals induced by hormones, neurotransmitters, lymphokines, and stress inducers are integrated into downstream messengers (7). These interactions are mediated by protein kinases that directly phosphorylate the erbB receptors, thereby affecting their kinase
activity or endocytotic transport. One type of trans-regulatory mechanism involves the activation of G-protein–coupled receptors (GPCRs), such as those for lysophosphatidic acid, thrombin, and endothelin. Agonists of GPCRs may result in a net increase of phosphorylation of erbB1 and erbB2. By a poorly defined mechanism, these agonists can also activate matrix metalloproteinases, which then cleave membrane-tethered erbB ligands, such as heparin-binding EGF, thereby freeing them to bind to erbBs. Activation of GPCRs may also activate Src family kinases, leading to phosphorylation of tyrosine residues on the intracellular domains of ErbB. These activities can subsequently trigger events downstream of ErbB1, possibly contributing to the mitogenic potential of heterologous agonists. Furthermore, interconnections between other signaling pathways help to integrate and coordinate cellular responses to extracellular stimuli.

The erbB family and related signaling network provide enormous signaling diversity at many levels, including ligand specificity, receptor partnering, scaffolding sites for effector signaling proteins, and substrate specificity for their kinase activities, receptor degradation, and integration of heterologous signals. Diversity among types of cells and tissues also exists, depending on the expression and preferred stoichiometry for interactions of the receptors and ligands. In sum, erbB receptors couple to specific downstream pathways with differing efficiencies, thereby affording an astonishing range of signaling possibilities. The particular cellular response to erbB stimulation is a function of the cellular context, as well as the specific ligand and erbB dimer. This has been shown best for mitogenic and transforming responses; homodimeric receptor combinations are less mitogenic and transforming than the corresponding heterodimeric combinations, and erbB2-containing heterodimers are the most potent complexes.

erbB in Cancer

In many different cancer cell types, the erbB pathway becomes hyperactivated or dysregulated by several mechanisms, including overproduction of ligands, overproduction of receptors, or constitutive activation of receptors. The particular mechanism by which erbB becomes hyperactivated may be very important in determining overall prognosis and guiding specific treatment.

erbB1  erbB1 is expressed on normal cells at levels ranging from 20,000 to 200,000 receptors per cell (20, 21). However, receptor levels can be much higher in malignant cells. Amplification of growth-factor receptor genes is one mechanism by which tumor cells can increase the number of cell-surface receptors. erbB1 gene amplification has been observed in several cancers, such as brain malignancies and non–small-cell lung cancer (NSCLC) (7, 21). In some renal-cell carcinomas and other malignancies, erbB1 may also be overexpressed in the absence of gene amplification owing to a variety of mechanisms, including mutations that increase erbB1 transcription, mRNA translation, or stability of the protein.

Many malignancies and cancer cell lines, especially carcinomas (Table 1), overexpress erbB1 (21). Increased erbB1 expression has also been reported in
TABLE 1  Malignancies overexpressing wild-type and mutated forms of erbB receptors

<table>
<thead>
<tr>
<th>erbB1</th>
<th>erbB2</th>
<th>erbB3</th>
<th>erbB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (14%–91%)∗</td>
<td>Breast (10%–37%)∗</td>
<td>Breast</td>
<td>Breast</td>
</tr>
<tr>
<td>Ovary (30%–75%)∗</td>
<td>Ovary (20%–32%)∗</td>
<td>Ovary</td>
<td>Ovary∗</td>
</tr>
<tr>
<td>Renal (50%–90%)</td>
<td>Renal (24%–40%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung (NSCLC) (40%–80%)∗</td>
<td>Lung (NSCLC) (3%–56%)∗</td>
<td>Lung (NSCLC)</td>
<td>Lung (NSCLC)</td>
</tr>
<tr>
<td>Head and neck (squamous) (30%–75%)∗</td>
<td>Head and neck (squamous) (32%–62%)∗</td>
<td>Head and neck</td>
<td>Head and neck</td>
</tr>
<tr>
<td>Colorectal (25%–77%)</td>
<td>Colorectal (7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas (30%–50%)∗</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glioma (40%–50%)∗</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder (31%–48%)∗</td>
<td>Bladder (7%–36%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagus∗</td>
<td>Esophagus (13%–73%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach∗</td>
<td>Stomach (5%–55%)∗</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>Prostate∗</td>
<td>Prostate</td>
<td>Prostate</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Melanoma</td>
<td>Melanoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Thyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial∗</td>
<td>Endometrial∗</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin (squamous cell)</td>
<td>Skin (squamous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung (small cell)</td>
<td>Lung (small cell)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical∗</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcomas</td>
<td></td>
<td></td>
<td>Chronic myelogenous leukemia</td>
</tr>
</tbody>
</table>

∗Clinical studies have linked overexpression and/or mutation of this erbB receptor to a worse prognosis.

melanoma and meningioma. The level of erbB1 expression and activity has been shown to vary widely within a tumor type, but this variability may be due, in part, to differences in detection methods. Table 2 illustrates the range of erbB1 overexpression observed in different studies for a given tumor type. erbB1 overexpression is associated with a higher grade, higher proliferation, or reduced survival in a variety of cancers including NSCLC, squamous-cell carcinoma of the head and neck (SCCHN), and carcinomas of the ovary and breast, among others. However, the data regarding the predictive value of erbB1 expression within a given tumor type are conflicting.

Several variants of the erbB1 receptor have been identified in many malignancies. The most common variant is the mutated erbB1 receptor EGFRvIII, which
TABLE 2  Selected antibodies to erbB1 in clinical development

<table>
<thead>
<tr>
<th>Agent (developer)</th>
<th>Class</th>
<th>Phase of development</th>
<th>Target</th>
<th>Pertinent clinical results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab (ImClone Systems)</td>
<td>Human recombinant antibody (chimera)</td>
<td>III</td>
<td>Binds to external domains of erbB1</td>
<td>Nonlinear pharmacokinetics with erbB1 saturation at doses &gt; 200 mg/m^2; T_{1/2} in plasma ≈ 7 days. Major toxicities: folliculitis and/or acneiform rash (80%); severe allergic reactions (4%). Human antichimeric antibodies, which are occasionally neutralizing, reported in 3% of patients. Early development in combination with chemotherapy or radiation. Colorectal cancer (irinotecan-refractory): combined with irinotecan, 22.5% major response rate; single agent, 10% major response rate. Pancreatic cancer (untreated): combined with gemcitabine, 12% major response rate. Phase III studies in head and neck and colorectal cancers in progress.</td>
</tr>
<tr>
<td>EMD72000 (Merck KGa)</td>
<td>Humanized IgG1κ antibody</td>
<td>I/II</td>
<td>Binds to the external domain of erbB1</td>
<td>Major toxicity: rash. Major activity in colorectal cancer in phase I.</td>
</tr>
<tr>
<td>ABX-EGF (Abgenix)</td>
<td>Human IgG2κ antibody (fully human)</td>
<td>II</td>
<td>Binds to external domains of erbB1</td>
<td>Major toxicity: rash. At the recommended phase II dose (2.5 mg/kg/week), receptor-mediated clearance is saturated and rash occurs in 100% of patients. Major activity in colorectal (10% major response rate) and renal cancers. Phase II studies in prostate, renal, colorectal, and lung cancers in progress.</td>
</tr>
<tr>
<td>MDX-447 (Medarex)</td>
<td>Bispecific monoclonal antibody</td>
<td>II</td>
<td>Binds to both erbB1 and CD64 on neutrophils and monocytes</td>
<td>Evidence of immunological activity, skin toxicity, and biological responsiveness in phase I. Phase II studies in progress.</td>
</tr>
</tbody>
</table>

is caused by deletion of exons 2 to 7 and subsequent loss of amino acid residues 6 through 276 in the extracellular domain. EGFRvIII is not found in normal tissues but is expressed on the cell membrane in certain tumors, including ~50% of gliomas. EGFRvIII has also been detected in medulloblastoma, in carcinomas of the prostate, breast, ovary, and stomach, and in NSCLC in varying frequencies, suggesting broad clinical relevance (21). This variant possesses a constitutively activated TK that may result in ligand-independent transformation of cell lines, although the mutation results in the deletion of a part of the extracellular domain that renders the receptor incapable of ligand binding and dimerization (20). Specific monoclonal antibodies (MAbs) have been isolated that can help to detect this
variant on tumor cells using immunohistochemistry. The use of such MAbs permits identification of EGFRvIII in certain tumors that may not bind MAbs against wild-type erbB (22, 23). The tumor-specific expression of this variant, coupled with the rapid internalization of EGFRvIII-MAb complexes, suggests that anti-EGFRvIII–targeted therapy using MAbs or immunoconjugates may be useful against tumors expressing this variant (23).

Although both overexpression and structural alterations of erbB1 occur commonly in human malignancies, particularly carcinomas, in vitro studies suggest that overexpression of the normal receptor leads to transformation only in the presence of a ligand, and expression of EGF-like ligands often accompanies erbB1 overexpression in primary tumors. Autocrine stimulation of the erbB1 receptor by production of EGF and TGF-α from the tumor cells can complete the feedback loop by binding to its own receptors. Therefore, tumor cells may require a functional autocrine loop for continued survival rather than increased expression of erbB1 alone.

In order to metastasize, tumor cells must complete several essential processes. These include stromal and vascular invasion, embolization, survival in the circulation, arrest in a distant capillary bed, and extravasation into and multiplication in organ parenchyma. erbB1 has been implicated in several pathways that affect tumor-cell survival and apoptosis, angiogenesis, motility, and invasion. Therefore, inhibition of EGFR activity could affect multiple aspects of tumor growth, progression, and metastasis.

**erbB2** Both in vitro and animal studies have clearly indicated that erbB2 overexpression plays a pivotal role in oncogenic transformation and tumorigenesis (24, 25). Transfection of the erbB2 gene into human breast and ovarian tumor cell lines increases DNA synthesis, tumorigenicity, and metastatic potential. Furthermore, the growth of tumors and human breast cancer cell lines overexpressing the erbB2 receptor is inhibited by MAbs directed at the receptor. erbB2 amplification/overexpression has been detected in subsets of a wide range of human cancers. When the erbB2 gene is amplified and the erbB2 receptor overexpressed, it is very likely that this overexpression contributes significantly to tumor development or progression. When only elevated levels of erbB2 protein are found with no erbB2 gene amplification, it is not clear whether these levels of protein are involved in the development or progression of the tumor. It is important to recognize the importance of detection methods and their interpretation in determining the percentage of tumors scored as positive for erbB2 protein expression.

The association of erbB2 expression with cancer has been demonstrated best in breast cancer, in which overexpression correlates with tumor size, spread of the tumor to lymph nodes, high grade, high percentage of S-phase cells, aneuploidy, and lack of steroid hormone receptors. Evidently erbB2 confers a strong proliferative advantage to tumor cells (24, 25). Other malignancies in which erbB2 gene amplification and/or protein overexpression are found include ovarian, endometrial, cervical, gastric, colorectal, bladder, kidney, pancreatic, and thyroid
carcinomas, as well as SCCHN and mesenchymal malignancies. Paradoxically, a higher degree of erbB2 overexpression is reported in early forms of breast cancer (such as ductal carcinoma in situ) than in more advanced invasive carcinomas, suggesting that alterations in erbB2 alone are insufficient for breast tumor progression from a relatively benign to a more malignant phenotype (24, 25). The structure of overexpressed erbB2 protein is the same as that found in normal cells; no mutational change in the erbB2 gene product has been identified in human cancers (26). Overexpression of erbB2 on the cell surface appears to lead to constitutive activation of erbB2 homodimers without the need for ligand binding, resulting in unregulated cell growth and oncogenic transformation.

Amplification of the erbB2 gene is a prerequisite of erbB2 protein expression in most tumors (24, 27). However, the erbB2 gene is not amplified in a small percentage of tumors that overexpress erbB2 mRNA or protein. In these rarer cases, erbB2 protein overexpression may result from transcriptional or post-transcriptional dysregulation. The identification of erb2 amplification by fluorescence in situ hybridization (FISH) has now received regulatory approval in the United States and elsewhere to predict which breast cancer patients are at high risk for recurrence and disease-related death following definitive local treatment. Many investigations are attempting to relate erbB2 amplification status (as determined by FISH) to benefit from therapies targeting erbB2. Accumulating data indeed indicate that erbB2 gene amplification by FISH can identify patients who might benefit from more aggressive therapy (7).

erbB3 AND erbB4 The catalytically inactive member of the erbB family, erbB3, is expressed in several epithelial cancers, but there is no evidence for gene amplification, and overexpression is limited. Coexpression of erbB3 and erbB4 with other erbB family members appears to improve the predictive power for prognosticating the overall course for certain tumor types; however, the roles of these receptors in cancer are not well understood.

RATIONALE AND TREATMENT STRATEGIES FOR TARGETING THE erbB1

The development of therapeutics targeting erbB2, particularly trastuzimab (Herceptin®, Genentech Inc., South San Francisco, CA), has been reviewed elsewhere (7). This section is limited to a discussion of therapeutics targeting erbB1. The coexpression of erbB and ligands at tumor sites allows erbB activation via autocrine/paracrine mechanisms. In support of the operational nature of these signaling pathways in erbB-expressing tumor cells, interruption of signaling with various erbB inhibitors has been shown to inhibit tumor proliferation in vitro and in vivo (28–33). These observations, coupled with (a) the ability to identify erbB-expressing human tumors from patients, (b) the association of erbB expression, particularly erbB1, with poor prognosis, and (c) the lack of a critical physiologic role of erbB (specifically erbB1) in healthy adults, have all suggested that this
network is an ideal target for novel therapeutic strategies. Over the past decade, it has been recognized that antibodies and small molecules (drugs) can be used to perturb erbB1 signaling at the cellular level, thereby inhibiting cell growth and promoting cell death. Because erbB1 is an integral component of critical signaling pathways involved in regulating tumor growth, a rational approach to cancer therapy is to block the function of the receptor and thus inhibit cell proliferation and tumor progression. Strategies to target erbB1 for anticancer therapy include the following: (a) MAb directed against the erbB1 receptor to block binding of EGF and TGF-α activated cell growth; (b) synthetic erbB1 TK inhibitors that act directly on the cytoplasmic TK domain of the receptor, preventing signal transduction and cell proliferation; and (c) ligand conjugates that bind specifically to erbB1 and deliver a lethal payload following ligand-toxin internalization (21). This review discusses the first two approaches, which are more developed at present.

Monoclonal Antibodies

HUMANIZED ANTIBODIES A number of MAbs recognize the extracellular domain of erbBs, particularly erbB1 and erbB2. Table 2 summarizes relevant features of selected MAbs targeting erbB1. Those directed against erbB1 compete for ligand binding, induce erbB dimerization and internalization, and inhibit ligand-stimulated TK activity, downstream signaling, and tumor growth (28–35). erbB is then degraded and downregulated. Many anti-erbB1 MAbs are more effective in vivo than in vitro, possibly because of the induction of antiangiogenic activity and/or enhancement of immune effector activity. The ability to induce receptor dimerization and downregulation from the cell surface and to block the receptor's catalytic function has been best characterized for the 528 mouse IgG2a and the 225 mouse IgG1, as well as the 225 humanized MAb, which led to the development of cetuximab (36). In erbB1-dependent tumor cells, this approach inhibits erbB1 signaling, leading to cell-cycle arrest and/or cell death (37, 38). In addition to blocking autocrine erbB1 signaling, it has been proposed that erbB1-targeting MAbs may recruit Fc receptor-expressing immune effector cells, leading to antibody-dependent cellular cytotoxicity and tumor eradication. In one study, less complete inhibition of A431 tumor growth was observed with 225 F(ab′)2 than with the bivalent 225 MAb, which suggests that, in addition to kinase blockade, immune mechanisms contribute to the antitumor activity of intact 225 MAb. The results of a phase I trial with the erbB1 mouse MAb 225 MAb showed selective antibody localization in NSCLC that had not been prescreened for erbB1, suggesting that the differential expression of erbB1 in tumor versus normal tissues can provide a therapeutic window for cancer syndromes with a high prevalence of detectable receptor expression (21, 28–33, 39–42). Cetuximab (IMC-C225), a chimeric humanized version of 225 MAb (Erbitux®, ImClone Systems Inc., New York, NY) was generated to avoid the host's immune response against mouse antibodies. Interestingly, cetuximab's binding affinity (Kd = 1–2 × 10^{-10} M) is approximately tenfold greater than those of the natural ligands (EGF and TGF-α) and the parental murine MAb (21). Cetuximab binding blocks EGF-induced activation, autophosphorylation, and
internalization of erbB1. The MAb arrests cells in the G1 phase of the cell cycle, which is associated with increased levels of p27\(^{Kip1}\) (21). Cetuximab and similar MAbs, such as EMD72000 (Merck KGA, Darmstadt, Germany), also inhibit the growth of erbB1-expressing cancers in vitro, reduce tumor volume, and/or increase survival in mice with a wide range of erbB1-expressing human cancers. These effects have also been associated with apoptosis. Furthermore, these MAbs enhance the effects of radiation and various chemotherapy agents in vitro and in vivo. These agents include platinating (e.g., cisplatin), DNA-intercalating (e.g., doxorubicin), and antimicrotubule (e.g., paclitaxel, docetaxel) agents (21, 28–42).

It has been proposed that in order to produce a significant antitumor response, erbB1 must be saturated with the MAb. Receptor saturation has generally been assumed to be achieved in vivo when the systemic elimination pathways for the MAb are saturated, at which point internalization of the MAb-erbB1 complexes becomes the principal mechanism of MAb clearance. The results of phase I studies indicate that cetuximab pharmacokinetics are nonlinear and saturation of elimination pathways occurs at intravenous doses of 200 and 400 mg/m\(^2\). The higher dose was associated with zero-order clearance during the first 96 h of the infusion and the half-life of the MAb was estimated to be 7 days (21). Cetuximab has principally been developed for weekly administration as a component of multi-agent regimens, since preclinical studies indicated that the predominant antitumor effect of therapeutics targeting erbB1 is delayed tumor growth, which may not impact the disease course of patients with advanced malignancies as profoundly as tumor regression does. The most common toxicity has been an acneiform rash or folliculitis involving the face, upper chest, and back, which occurred in 80% of patients and probably relates to the prominent roles of erbB1 and EGF-like ligands in epidermal tissues (27–42). Severe allergic reactions occurred in ~4% of patients, with most occurring within minutes of the first infusion. Approximately 3% of patients had detectable human antichimeric antibodies, which were occasionally neutralizing (21). Overall, there was little evidence of significant production of human antichimeric antibodies in response to cetuximab treatment, and such antibodies have not precluded repetitive treatment. Prominent antitumor activity has been noted in patients with several types of advanced malignancies in phase I and II trials of cetuximab combined with other therapeutic modalities, as well as in limited studies of cetuximab as a single agent in colorectal cancer, as outlined in Table 1 (37–42).

**HUMAN ANTIBODIES** A fully humanized IgG2\(\kappa\) Mab specific to erbB1 (ABX-EGF, Abgenix, Inc., Freemont, CA) has also been generated using a XenoMouse\(^\circledR\) technology, in which human immunoglobulin genes were introduced into mice engineered to lack functional mouse immunoglobulin genes. Unlike humanized MAbs—which are constructed by implanting the complementary-determining regions of the mouse antibody into the human immunoglobulin framework, still contain ~5%–10% mouse protein sequences, and may still be immunogenic—this human MAb does not contain murine protein sequences (43, 44). The full
humanization would be expected to result in no immunogenicity and a slower clearance rate of the MAb as compared with mouse or mouse-derived MAbs, thus allowing repeated antibody administration in immunocompetent patients. The ABX-EGF MAb binds erbB1 with high affinity ($K_d = 5 \times 10^{-11}$ M), thereby blocking binding of both EGF and TGF-α, and inhibits EGF-dependent tumor-cell activation and proliferation (44, 45). ABX-EGF completely prevents the formation of human epidermoid carcinoma A431 xenografts in athymic mice (43, 44). More importantly, ABX-EGF without concomitant chemotherapy completely eradicates well-established A431 erbB1-overexpressing tumors, which do not recur. Human pancreatic, renal, breast, and prostate tumor xenografts, which express different levels of erbB1, were also inhibited by ABX-EGF treatment. The number of erbB1 molecules per cell correlated with the degree of tumor growth inhibition; MAb had no effect on tumors that did not express erbB1.

Preliminary results of early clinical evaluations of ABX-EGF administered weekly in patients with various malignancies likely to express erbB1 indicate that the ABX-EGF is well tolerated at doses predicted to induce antitumor activity based on modeling of preclinical data (45, 46). The predominant toxicity is a transient acneiform rash and human antihuman antibodies have not been detected. Disease-directed evaluations have recently begun in patients with advanced NSCLC and renal, prostate, and colorectal cancers, and intriguing activity has been observed to date in the most mature studies in renal and colorectal cancer patients (45–47).

**BISPECIFIC ANTIBODIES** Bispecific antibodies, largely directed to erbB1, have dual specificity because they have two different antibody-binding regions. One is specific for the erbB1 receptor and the other is engineered to bind to an immunologic effector cell (48). The result is an antibody that binds to erbB (preventing signaling and related effects) and also enhances the host’s cytotoxic effector mechanisms. Antibodies specific for erbB1 and the immunoglobulin receptor CD64, which is found on monocytes and neutrophils, include MDX-447 (Medarex, Inc., Princeton, NJ). Both bispecific antibodies have been shown to reduce tumor growth and enhance immunologically mediated cytotoxicity. The results of phase I evaluations show immunologic activity, good tolerability, and biologic responses, and phase II disease-directed studies are ongoing (48).

**SMALL MOLECULES TARGETING erbB1 TYROSINE KINASE** Another approach to inhibiting erbB1 involves small molecules designed to inhibit RTK activity, erbB phosphorylation, and critical signaling downstream (21, 28, 33, 49, 50). Hypothetically, this approach could inhibit signaling mediated by ligands, as well as signaling that is independent of growth factors. In contrast to MAbs, such agents may also inhibit ligand-independent signaling due to constitutively active mutant receptors (e.g., EGFRvIII), but the relative merits of these modalities
for inhibiting such mutant receptors are not clear. This strategy has involved the random screening from natural or synthetic compound libraries of small molecules with molecular weights of 300–400 Da that compete with the Mg-ATP binding site of the catalytic domain of the erbB1-TK (21, 28–33, 49, 50).

The anilinoquinazolines have clearly set the boundaries for defining potency and specificity of inhibitors of erbB1 receptor TK (49, 50). Members of this class have demonstrated potencies as low as 6 $\mu$M against the erbB1 TK, with almost total specificity relative to other receptor and intracellular TKs, as well as potent, specific inhibition of all EGF-mediated processes in viable cells. More recently, the quinazoline series of inhibitors has been expanded to include fused tricyclic derivatives, such as imidazoloquinazolines, pyrroloquinazolines, and pyrazoloquinazolines. These compounds have IC$_{50}$ values against the isolated enzyme that are in the picomolar and low nanomolar ranges for inhibition of EGF-mediated receptor autophosphorylation. Tables 3 and 4 list several erbB1 TK inhibitors in clinical development, and this section discusses the salient preclinical and clinical characteristics of several of these compounds. The anilinoquinazolines, gefitinib (ZD1839; Iressa®; AstraZeneca, Wilmington, DE) and erlotinib (OSI-774; Tarceva®; OSI Pharmaceuticals, Uniondale, NY) are in the most advanced stages of clinical development. Both compounds inhibit the purified erbB1-TK enzyme in vitro with IC$_{50}$ values in the low nanomolar range (51–54). Because of the high intracellular concentrations of ATP, much higher concentrations of the reversible erbB1-TK inhibitors are required to continuously block erbB1 phosphorylation in intact cells than in acellular experimental systems, which served, in part, as the rationale for developing of irreversible TK inhibitors such as CI-1033 (Pfizer, Inc., Groton, CT), EKB-569 (Wyeth-Ayerst, Philadelphia, PA) as these compounds bind irreversibly to the receptor, competing effectively with high concentrations of ATP for receptor binding. The ~80% homology between the erbB1 and erbB2 TKs has allowed the generation of the RTK inhibitors, CI-1033, EKB-569, and GW572016 (Glaxo SmithKline, Philadelphia, PA), which inhibit multiple erbB receptor families (21, 55–57). This review does not discuss specific small-molecule inhibitors of erbB2 TK, which are also under development.

**Gefitinib**

Gefitinib (ZD-1839; Iressa®) was initially shown to selectively inhibit erbB1 TK activity and EGF-dependent proliferation of KB SCCHN cells with IC$_{50}$ values of 23 and 80 nM, respectively (Table 3) (22, 29–37, 52–54). However, it was at least 100-fold less active at inhibiting other kinases. The agent exerts various effects on tumor cells that express erbB1, such as blocking receptor autophosphorylation, inducing cell-cycle arrest, and reducing cell proliferation. Tumor growth inhibition is associated with a dose- and time-dependent upregulation of the cyclin-dependent kinase inhibitor p27Kip1, which may account for cell-cycle arrest in the G$_1$ phase. Gefitinib also inhibits angiogenesis. Tumor growth inhibition has been the predominant therapeutic effect of gefitinib in vitro and in vivo, but DNA fragmentation indicative of apoptosis and tumor regression has been noted in several
**TABLE 3** Selected small-molecule reversible inhibitors of erbB receptor tyrosine kinase activity in clinical development

<table>
<thead>
<tr>
<th>Agent (developer)</th>
<th>Chemical class</th>
<th>Target and mechanism of action</th>
<th>Pertinent clinical results</th>
</tr>
</thead>
</table>
| Gefitinib (ZD1839; AstraZeneca) | Quinazoline | Competitively inhibits ATP binding in the internal TK domain of erbB1 | Phase I studies evaluated both intermittent (once-daily for 14 days every 28 days) and continuous (once daily, uninterrupted treatment) schedules Major toxicities: rash, diarrhea IC₅₀ against erbB1 TK activity in vitro is 0.020 µM | Phase II studies in previously treated patients with NSCLC:  
  - Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL)-1 Study (Japan/Europe; no screening for erbB1): 250 vs. 500 mg/day, major response rate, 18.4% vs. 19%  
  - IDEAL 2 (USA; no screening for erbB1): 250 vs. 500 mg/day, major response rate, 12% vs. 9%. Stable disease in 31% vs. 27%. Lung cancer symptoms respond in 95% and 71% of patients who had major responses and stable disease, respectively. Accelerated regulatory approval in May 2003 for patients with advanced NSCLC following failure of platinum- and docetaxel-based therapies  
  - Phase III study (no screening for erbB1):  
    - Iressa NSCLC Trial Assessing Combination Treatment (INTACT)-1 and -2: Chemotherapy, consisting of paclitaxel/carboplatin or gemcitabine/oxiplatin, plus placebo or gefitinib 250 or 500 mg/day; combined therapy for 6 courses followed by maintenance gefitinib or placebo: no differences in response rates, progression-free survival, or overall survival |
| Erlotinib (OSI-774; OSI/Genentech/Roche) | Quinazoline | Competitively inhibits ATP binding to the ATP binding site in the internal TK domain of erbB1 | Phase I studies evaluated continuous (once-daily) dosing schedule Major toxicities: rash and diarrhea IC₅₀ against erbB1 TK activity in vitro is 0.002 µM | Phase II studies: activity noted in previously treated NSCLC, ovarian, and head and neck cancers, with major responses in 11%, 10%, and 13%, respectively Phase III studies in NSCLC and pancreatic cancers, as well as broad phase II evaluations, are ongoing Major responses in brain tumors in phase I studies |
| GW572016 (GlaxoSmithKline) | Quinazoline | Competitively inhibits ATP binding to the ATP binding site of the internal TK domains of erbB1 and erbB2 | Phase I studies evaluated continuous (once daily uninterrupted) schedule Major responses in breast cancer in phase I studies Major toxicities: rash, nausea and vomiting, diarrhea Phase II evaluations ongoing |
TABLE 4  Selected small-molecule irreversible inhibitors of erbB receptor tyrosine kinase activity in clinical development

<table>
<thead>
<tr>
<th>Agent (developer)</th>
<th>Chemical class</th>
<th>Target and mechanism of action</th>
<th>Pertinent clinical results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-1033 (Pfizer)</td>
<td>Quinazoline</td>
<td>Irreversibly binds to cysteine residues in the ATP binding site of the internal TK domains of erbB1, erbB2, and erbB4 and prevents transmodulation of erbB3 Particularly potent against erbB1 and erbB2 TK</td>
<td>Phase I studies evaluated various once-daily intermittent and continuous treatment schedules Major toxicities: rash, diarrhea, nausea, vomiting. Also, dose-related myelosuppression and hypersensitivity reactions in studies evaluating intermittent treatment (e.g., once weekly) $T_{1/2} = 4-6$ h Phase II studies ongoing in ovarian, breast, and non-small-cell lung cancers</td>
</tr>
<tr>
<td>EKB-569 (Wyeth Ayerst)</td>
<td>Quinazoline</td>
<td>Irreversibly binds to cysteine residues in the ATP binding site of the internal TK domains of erbB1 and erbB2 IC$_{50}$ = 1.33 nM against erbB1 TK in vitro and 15 nM against cells that overexpress erbB1 Striking protection against polyp formation in APC$^{Min/+}$ mice when administered with sulindac</td>
<td>Phase I studies evaluated once-daily continuous (for 21 days) schedule Major toxicities: skin rash, diarrhea, mucositis, nausea $T_{1/2} = 20-24$ h Development in colorectal and pancreatic cancers as a single agent and in combinations</td>
</tr>
</tbody>
</table>

There is also ample experimental evidence that the autocrine activation of erbB1 signaling is important in breast cancer cells with acquired resistance to tamoxifen. Breast cancer cell lines with acquired tamoxifen resistance and impressive sensitivity to gefitinib have also been described, and these cells are more sensitive to gefitinib than tamoxifen-sensitive parental cells (41, 42). Because erbB1 and other erbB family members form heterodimers, particularly with erbB2, resulting in receptor transmodulation, it is likely that gefitinib also interferes with erbB1-driven transactivation of other erbB receptor types. Furthermore, low gefitinib concentrations are effective at inhibiting growth of erbB2-overexpressing breast cancer cells that also express erbB1 (41, 42). In fact, gefitinib exhibited greater growth
inhibition than the erbB2-targeted MAb trastuzumab, and the agent, at higher concentrations, was effective against trastuzumab-resistant erbB2 breast cancer cells. Such results provide a rationale for the combined use of therapeutics targeting both erbB1 and erbB2.

Mounting evidence suggests that gefitinib and other erbB1-targeted therapeutics may be useful in cancer prevention (58). Gefitinib, administered on an oral, once-daily, uninterrupted schedule, is undergoing clinical evaluations (Table 3). Most studies to date have been conducted in patients whose tumors have not been screened for erbB1 or potential determinants of response. Antitumor activity has been noted in patients with NSCLC, as well as breast, brain, head and neck, and prostate cancers. In May 2003, gefitinib received accelerated regulatory approval in the United States as monotherapy treatment (250 mg/day) for patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapy.

Erlotinib

Erlotinib (OSI-774; Tarceva®) is a reversible, ATP-competitive inhibitor of the erbB1 TK, with an IC$_{50}$ value of $\sim 2$ nM and $\geq 1000$ times the selectivity of pp60$\text{src}$, pp145$\text{c-abl}$, insulin receptor, and insulin-like growth factor–1 receptor TKs (21, 28–32, 54). The IC$_{50}$ for inhibition of EGF-mediated receptor autophosphorylation of HN5 human head and neck cancer xenografts was 20 nM, and EGF-dependent mitogenesis and proliferation was reduced by 50% at comparable concentrations. Similar results were obtained in the DiFi colorectal carcinoma and the MDA-MB-468 breast carcinoma cell line. Erlotinib can induce cell-cycle arrest with an accumulation of cells in G$_0$/G$_1$, loss of the hyperphosphorylated form of the retinoblastoma protein, and accumulation of p27$\text{kip}1$. The agent also induces apoptosis, but higher drug concentrations are generally required. The combination of apoptosis and cell-cycle arrest lends further support to the concept that programmed cell death and differentiation may be the principal cellular mechanisms by which erbB-targeted TK therapeutics function.

Erlotinib showed impressive antitumor activity both in vitro and in vivo. In preclinical studies, OSI-774 was well tolerated at doses of 10–200 mg/kg daily, had good oral bioavailability, and had a pharmacokinetic profile that was conducive to once-daily administration (54). Erlotinib showed significant in vivo activity: An oral dose of 10 mg/kg reduced HN5 xenografts by 50%, and a 50-mg/kg dose nearly achieved tumor stasis. Similar results were obtained against erbB1-overexpressing A431 xenografts. In clinical trials to date, in which the agent is administered once daily on an uninterrupted schedule, antitumor activity is observed in patients with NSCLC and cancers of the breast, head and neck, ovary, and brain (21).

GW572016, a Combined erbB1 and erbB2 TK Inhibitor

GW572016, a 6-thiazolyquinazoline that reversibly inhibits the phosphorylation of erbB1 and erbB2 and downstream MAPK in a dose-dependent manner, has
demonstrated potent tumor growth inhibitory activity in vitro (IC\textsubscript{50} values below 0.15 \(\mu\)M) and appears selective for tumor cells relative to normal cells (56). In vivo studies showed antitumor activity against erbB1-overexpressing head and neck carcinoma and erbB2-overexpressing breast carcinoma xenografts. Clinical evaluations of GW572016 administered on a once-daily uninterrupted schedule are ongoing in tumor types such as breast and colorectal cancers, which are likely to express both erbB1 and erbB2. Preliminary activity has been reported in patients with advanced breast cancer (60).

Irreversible Inhibitors of Multiple erbB Receptor Tyrosine Kinases

EKB-569 and CI-1033 are composed of chemical moieties that form covalent bonds within the RTK domain, resulting in irreversible receptor binding and sustained TK inhibition in vitro. This feature may also circumvent drug binding competition due to high intracellular ATP concentrations. However, the rates of receptor turnover and drug clearance in vivo are probably important factors to consider when comparing the merits of reversible and irreversible inhibitors of RTK. The optimal use of reversible erbB TK inhibitors mandates relevant plasma concentrations and/or agents with relatively long half-lives to keep the target suppressed, whereas the use of irreversible compounds would require that plasma concentrations be attained only long enough to briefly expose the receptors to the drug, which would then permanently suppress kinase activity. Like cetuximab, the erbB1-selective, reversible inhibitors appear to induce regression of tumors with either high or low erbB1 expression, suggesting that high erbB1 levels are not necessarily a predictor of tumor response to erbB1-targeting therapeutics.

CI-1033  CI-1033 (Pfizer, Inc., Groton, CT) is a 4-anilinoquinazoline that irreversibly binds within the ATP-binding pocket of erbB TK and inhibits both activation and downstream signaling emanating from erbB1, erbB2, erbB3, and erbB4 (21, 49, 50, 55). CI-1033 binds irreversibly with high affinity to all erbB family members, particularly erbB1 and erbB2. It inhibits isolated erbB1 TK activity with an IC\textsubscript{50} value of 1.5 nM and inhibits heregulin-mediated tyrosine phosphorylation in MDA-MB-453 human breast carcinoma, which expresses erbB2, erbB3, and erbB4, with an IC\textsubscript{50} value of 9 nM (50, 55). CI-1033 has been shown to inhibit erbB1 phosphorylation in A431 carcinoma and MDA-MB-468 human breast cancer cells, and it inhibits the growth of several human xenografts (50, 55). It also induces regression of well-established A431 tumors. The results of studies of long-term drug administration indicate that CI-1033 maintains tumor growth suppression for extended time periods without the emergence of drug resistance (49, 50, 55). The activity of the compound appears to be independent of dose fractionation; significant activity is obtained on dosing regimens ranging from once daily to once weekly (50, 55). In addition, CI-1033 enhances the cytotoxic effects of other therapeutic modalities (21). For example, the agent enhances the cytotoxic
effects of the topoisomerase inhibitors SN-38 and topotecan in vitro, possibly by interfering with a relevant drug-resistance mechanism. Phase I evaluations of CI-1033 administered once daily on various intermittent and noninterrupted schedules have been completed, and phase II disease-directed evaluations are ongoing (Table 4).

**EKB-569**

EKB-569 (Wyeth-Ayerst, Philadelphia, PA) is a 3-cyanoquinoline that binds covalently and irreversibly to erbB1 (Table 4). It is a potent inhibitor of a recombinant form of erbB1 TK (IC\(_{50}\) = 1.33 nM) and phosphorylation of erbB1 in cells (IC\(_{50}\) = 15 nM in cells that overexpress erbB1) (58, 59). Although >10 times more drug is required to inhibit other erbB TKs, including erbB2, EKB-569 is equipotent at inhibiting the growth of cells that overexpress erbB1 or erbB2. The effect is specific because 10- to 50-fold more drug is needed to inhibit the growth of cell lines that do not overexpress erbB1 or erbB2. Notable growth inhibition has also been observed in tumors derived from cells that overexpress erbB1 or erbB2, but EKB-569 does not inhibit the growth of tumors with low erbB expression. Consistent with its ability to irreversibly bind to erbB1 and erbB2, inhibition of receptor phosphorylation is sustained far longer than plasma levels of EKB-569. Furthermore, a combination of EKB-569 and the nonsteroidal anti-inflammatory agent sulindac provided a striking protection against colon tumor formation in a mouse model of familial adenomatous polyposis (>95% reduction in polyps), a surrogate for the development of colon cancer (59). Phase I evaluations of EKB administered once daily continuously and for 3 weeks every 4 weeks have been completed, and both phase II single-agent and combination evaluations are ongoing.

**CLINICAL DEVELOPMENT AND OBSTACLES AHEAD**

In clinical evaluations, inhibitors of erbB1 TK have demonstrated excellent tolerability, both as single agents and combined with other anticancer therapeutic modalities (Tables 2–4). Interestingly, the principal toxicity of both MAbs and small molecules directed against erbB1 is an acneiform and/or follicular skin rash, which may be a pharmacodynamic marker of clinical benefit. Consistent antitumor activity with reversible small-molecule inhibitors of RTK and MAbs alike has been noted in patients with NSCLC. Interestingly, however, notable antitumor activity has occurred in patients with colorectal cancer following treatment with MAbs but not the small-molecule TK inhibitors. The relative benefits conferred by irreversible erbB1 TK binding and inhibition of multiple erbB subfamilies will be known following clinical evaluations of CI-1033, EKB-569, and GW572016 in relevant tumor types. Nevertheless, rates of major tumor regression in nonrandomized clinical evaluations have been fairly low, which probably reflects the indiscriminate treatment of unscreened patients with tumors that may or may not possess the appropriate target or determinants for response. The failure to determine which patients are likely to benefit from such therapeutics may also explain why
randomized phase III studies have found no clear advantage of erbB1-targeted therapeutics combined with cytotoxic therapy in unscreened patients with NSCLC. It is important to note that the success of trastuzimab, specifically in increasing the survival of patients with breast cancer, would not have been appreciated if patients had not been screened before treatment for the principal target of trastuzimab, erbB2. Furthermore, since tumor growth delay (in contrast to tumor regression) appears to be the predominant benefit of erbB-targeted therapeutics in preclinical studies, particularly in tumors that are not exclusively driven by erbB dysregulation or overexpression, it will be increasingly necessary to select appropriate endpoints for phase II screening studies to appreciate and quantify drug-induced tumor growth delay. In practicality, however, some indication that the erbB inhibitors possess relevant clinical activity and can modify the natural history of disease progression will be needed before resource-intensive large randomized phase III studies are commenced. Adequately designed clinical trials will ensure that the usefulness of erbB-targeted therapeutics is correctly assessed, so that potentially useful agents are not rejected on the basis of poor performance with regard to an inappropriate clinical or biologic endpoint.

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