Central themes in medical practice are the diagnosis, prognosis and treatment of disease. Advances have been made in a number of malignancies including breast cancer, where new therapeutic strategies have significantly improved response rates, the disease-free interval and overall survival. However, complete responses to chemotherapy are achieved in only 10–20% of patients. Recent advances in the understanding of the cellular and molecular biology of cancer have led to the identification of oncogenes and tumor suppressor genes that influence the rate of tumor cell proliferation and cancer progression. These oncogenes represent important therapeutic targets and are currently being incorporated into the design of novel therapeutic approaches. This review emphasizes the role of the ErbB oncogenic receptor family, its effect on tumor biologic behavior and its role as a target for various therapeutic regimens.

Background & significance
Normal cells express various cellular receptors, including tyrosine kinase receptors such as ErbB (also referred to as human epidermal growth factor receptor (HER)) receptors, and nuclear receptors such as estrogen and progesterone receptors (ER and PR). Complex interactions between cellular receptors and their cognate ligands, which function by binding to, or interacting with, the cellular receptors, regulate the growth, development and proliferation of cells. Changes in these interactions contribute to uncontrolled cell growth and cancer (Figures 1 & 2) [1–12].

Determination of cell fate, in addition to rapid responses to extracellular cues, is critically mediated by soluble growth factors and their transmembrane receptors. The ErbB family of receptor tyrosine kinases (RTKs) and their ligands, soluble polypeptides of the epidermal growth factor (EGF)/heregulin (HRG) family are an example for this mechanism. By timely engagement of their growth factors at the cell surface, these receptors instigate a biochemical processes with a capacity to drive dramatic cellular transitions, such as proliferation and migration. Specifically, ligand binding of either receptor induces receptor homo- and heterodimerization. This in turn leads to receptor autophosphorylation and activation. Two distinct sets of ligands recognize ErbB1, B3 and B4, whereas a soluble ligand for ErbB2 has not been identified [13–37]. Of the ErbB receptors, ErbB3 is unique in that it is a ‘kinase-dead’ receptor. Thus, both ErbB3 and B2 cannot signal in isolation. However, both receptors can enhance and diversify signaling by EGF-like ligands, once they engage in heterodimers with ErbB1 and B4 [35–37]. Furthermore, ErbB2:B3 heterodimers represent the most mitogenic receptor complex, activating signaling pathways involved in regulating cell growth and survival [29]. ErbB3 and B4 mediate the biologic effects of HRG. ErbB1 (also referred to as the EGF receptor [EGFRI]) and ErbB2 are coreceptors for HRG. In fact, ErbB2 is a critical component in mediating HRG-induced breast cancer cell growth. Specifically, if ErbB2 is not available to form a heterodimer complex with other members of the ErbB family, then HRG activity is blocked and cell growth is decreased (Figure 3). Although lacking its own soluble ligand, ErbB2 is the preferred heterodimeric partner for other ErbB receptors, magnifying the amplitude and duration of intracellular signals by reducing the rate of growth factor dissociation from its cognate receptor (Figure 4) [38–49]. In addition, the major process that attenuates RTK signaling involves the sorting of ligand-activated receptors to endocytosis and subsequent degradation in lysosomes. However, endocytosis of ErbB2-containing heterodimers is relatively slow, and these complexes tend to recycle back to the cell surface [50–67].

With the exception of ErbB3, all ErbB receptor family members share a highly conserved cytoplasmic tyrosine kinase domain. Autophosphorylation of specific cytoplasmic tyrosine residues establishes binding sites for Src-homology (SH)-2 and phosphotyrosine binding domain-containing proteins, that, in turn, link to downstream effectors involved in cell proliferation.

Keywords: antibodies to ErbB receptors, ErbB receptor family, oncogenic receptors, targeted therapy, tyrosine kinase inhibitors
(mitogen-activated protein kinase [MAPK] or extracellular signal regulated kinase [Erk]1/2) and survival (phosphoinositide-3 kinase [P13K]/Akt) pathways [21,29,34,36].

Overexpression, gene amplification or mutations of ErbB1 are found in multiple human tumors, including cancers of the breast, head and neck and lung, with a particularly high incidence of overexpression in brain tumors of glial origin. Gain-of-function mutations within the kinase domain have recently been identified in a subclass of non-small cell lung carcinoma. Several tumors show co-expression of ErbB1 and its ligands, primarily transforming growth factor (TGF)α, in line with ligand-dependent malignant transformation. Overexpression of the ERBB1 gene has been implicated as a poor prognostic feature in various human malignancies including breast, head and neck, ovarian, bladder, and esophageal cancer. Furthermore, ErbB1 overexpression has been implicated as playing a role in mediating resistance to radiotherapy. In addition to wild-type ErbB1, cancer cells have also been shown to express various mutated ErbB1 molecules. The most common mutant, named EGFReIII, is one in which amino acids 6–273 (exons 2–7) of the extracellular domain are deleted. This in-frame deletion is common in glioblastomata and in several other types of cancer, including breast, ovarian, lung and medulloblastoma tumors. EGFReIII is constitutively active in a ligand-independent manner, and while its prognostic significance remains incompletely understood, it has been linked to resistance to radiation therapy [68–76].

Overexpression of the ERBB2 gene is frequently observed in human carcinomas. Examples include breast, ovarian and lung cancer, as well as tumors of the pancreas, colon, esophagus, prostate, endometrium, and cervix. The association of ErbB2 expression with disease parameters was best studied in the setting of breast cancers, where ErbB2 overexpression as a result of gene amplification occurs in 15–30% of invasive ductal carcinomas. The incidence of ErbB2 overexpression is higher in ductal carcinoma in situ (DCIS) of the comedo type. These observations, and the association of ErbB2 overexpression with tumor size, lymph node status, high grade, high percentage of S-phase cells, aneuploidy, and lack of steroid hormone receptors, imply that ErbB2 primarily confers a proliferative rather than an invasive advantage to tumor cells. The prognostic significance of ErbB2 overexpression in other tumors is not as well-established, but several reports suggest...
ErbB receptor targeted therapies – REVIEW

prognostic implications in pancreatic, ovarian, gastric and prostate cancer [77–82]. In addition to ErbB receptor overexpression, many cancers, notably breast cancer, also co-overexpress ErbB receptor ligands such as HRG and TGFα. HRG expression is also relevant to ER signaling and, therefore, anti-estroge therapies. Constitutive expression of HRG contributes to the progression of breast cancer cells from a hormone-dependent state, where ER plays a significant role in regulating tumor cell proliferation and survival, to a hormone-independent state. The hormone-independent state is generally associated with a poor prognosis and more aggressive tumor [85,88].

ErbB1 and B2 promote tumor growth and survival in a variety of epithelial tumors, where their expression or overexpression in some tumors correlates with a poor clinical outcome, making them attractive therapeutic targets [83–125]. However, clinical results with therapeutic compounds targeting these receptors have been mixed, implicating the need to understand the interplay between the ErbB receptors, their ligands, and other redundant signaling pathways contributing to the growth and survival of tumor cells.

ErbB receptors as targets for therapy
Due to the fact that ErbB proteins and their cognate ligands are intimately involved in a variety of epithelial malignancies, they have been targeted for a variety of therapeutic strategies, including the development of low-molecular-weight tyrosine kinase inhibitors, antireceptor monoclonal antibodies (mAbs), and drug and toxin conjugates of ligands and mAbs. Additional experimental approaches are under development, for example, gene therapy using an adenoviral protein to downregulate ErbB2, antisense strategies, and RNA aptamers that target the dimeric form of ErbB proteins. Several features make ErbB signaling particularly attractive for therapeutic intervention. The receptors, as well as their ligands, are available for extracellular manipulation, which avoids complications related to drug permeability across the plasma membrane. In addition, interventions at the receptor level, rather than at downstream effectors, offers higher selectivity (Figure 5).

Therapeutic mAbs and small molecule tyrosine kinase inhibitors targeting ErbB1 or B2 have been developed. Trastuzumab (Herceptin®) (Figure 6), a humanized anti-ErbB2 mAb, is approved for treating patients whose breast cancers overexpress the ErbB2 protein or demonstrate ERBB2 gene amplification (Table 1). In addition, gefitinib and erlotinib, small molecule tyrosine kinase inhibitors of ErbB1 (Table 2), are approved for third-line treatment of non-small cell lung cancer. The inability of mono-ErbB1 inhibitors, such as gefitinib and erlotinib, to demonstrate a survival advantage when added to first-line chemotherapy in metastatic non-small cell lung cancer raises questions
regarding the optimal use of these agents in the clinic and underscores the need to identify biomarkers to guide their clinical development.

The relatively low response rate observed when ErbB inhibitors are administered to patients who are selected solely on the basis of the overexpression of a single ErbB receptor is not unexpected given the complexity of ErbB signaling pathways. Most tumors of epithelial origin express multiple ErbB receptors and co-express one or more EGF-related ligands, suggesting that autocrine receptor activation plays a role in tumor cell proliferation and survival. As these ligands activate different ErbB receptors, it is possible that multiple types of ErbB receptor heterodimers are regulating cell growth and survival, a scenario that might influence response to an ErbB-targeted therapy [37]. The ligands present in the tumor microenvironment may determine the types of ErbB receptor dimers that are formed, thereby influencing the time course of membrane translocation, activation and internalization of the receptor and, ultimately, the type of signal generated [42]. Downstream signaling may be determined by the set of docking proteins that bind to the activated receptors. For example, ErbB3 contains six docking sites for PI3K. HRG stimulation of ErbB receptors causes activation of the PI3K pathway and phosphorylation of Akt [49,83]. These observations implicate PI3K/Akt in the signaling cascade that results from ErbB3 heterodimerization with overexpressed ErbB2 in breast cancer cells. Importantly, activation of PI3K/Akt promotes cell survival and enhanced tumor aggressiveness and resistance to chemo-, hormone, radiation, and biologic (for example, trastuzumab) therapy. Hence, inhibition of the Akt pathway may be very important for sensitizing tumor cells to a variety of therapeutic agents. Recent results suggest that ErbB receptors may be transactivated by other receptor classes such as G-protein-coupled receptors, cytokine receptors and insulin-like growth factor receptor (IGF-IR) [83].

The use of molecular markers to characterize tumors is critical toward selecting patients for tumor-targeted therapies, including those targeting ErbB receptors. In addition, profiling tumors based on molecular characteristics rather than histology alone will enable rational design of combination therapy using multiple tumor-targeted agents. Thus, use of the biologic markers allows the earlier identification of treatments and therapeutic agents likely to result in a clinically
positive outcome. In addition, use of the targeted therapy markers allows earlier identification of treatments and therapeutic agents that are not likely to result in a clinically positive outcome, sparing those patients who are not likely to benefit from potential toxicity [114–124].

**Antibodies to ErbB1 & B2**

mAbs to receptor tyrosine kinases such as ErbB1 and ErbB2 inhibit the tumorigenic growth of certain cancer cells, although the exact mechanism(s) responsible for their antitumor activities remain largely unknown. Anti-ErbB1 (EGFR) antibodies promote a slow endocytic process distinct from the rapid EGF-induced receptor internalization, and also inhibit ligand binding to the EGFR receptor. Combining mAbs that engage distinct epitopes significantly accelerates receptor degradation. In addition, combinations of mAbs are more effective than single Abs in inhibiting ErbB2 signaling in vitro and tumorigenesis in animals [119]. Early studies elucidated the tumor-inhibitory potential when mAbs directed at ErbB1 and B2 were used alone [89,95,106], with subsequent preclinical studies demonstrating that anti-ErbB mAbs appear to be more effective when combined with various chemotherapeutic agents [90,103,110,111,118,119]. The benefit of combining mAbs with chemotherapeutic agents has been demonstrated clinically using trastuzumab and cetuximab (C225), the latter being an anti-ErbB1/EGFR mAb for the treatment of breast and colorectal cancer, respectively. Two mechanisms have been implicated in ErbB-directed immunotherapy. The first involves mAb-mediated recruitment of natural killer cells to tumors. The second involves the blockade of ligand binding or receptor heterodimerization and accelerated receptor internalization [103], resulting in the inhibition of downstream proliferation and survival signaling pathways (e.g., PI3K/Akt, and MAPK/Erk1/2) [89]. The latter mechanism is particularly attractive because ligand-induced endocytosis and degradation of active RTKs is considered a major physiological process underlying attenuation of growth-promoting signals. Several studies reported cooperative effects of mAb combinations, whereas others found that bivalent, Fc-lacking versions of anti-ErbB mAbs inhibit tumorigenic growth in animals [95].

mAbs targeting the ErbB-receptor family include antibodies that prevent ligand binding and ligand-dependent receptor activation (for example, cetuximab), antibodies that interfere with ligand-independent receptor activation (for example, trastuzumab), and a new class of anti-ErbB receptor antibodies that prevent receptor heterodimerization (for example, 2C4 or pertuzumab) [127]. Anti-ErbB1/EGFR antibodies that interfere with ligand-dependent receptor activation have shown clinical activity in a variety of solid tumors including colon, head and neck, non-small cell lung cancer (NSCLC), and renal cell carcinomas. Single-agent clinical trials have been conducted in these tumor types and a substantial proportion of studies have also incorporated anti-EGFR mAbs into commonly used combination chemotherapy regimens. As expected, the clinical development of anti-EGFR antibodies is highly dependent on the tumor type being studied, with each tumor requiring...
different study endpoints, tumor-based chemotherapy regimens, and potential for integration with radiation therapy (RT).

To address the need for predictive biomarkers to identify patients more likely to respond to trastuzumab therapy, the authors analyzed breast cancer tissue obtained from patients treated with trastuzumab combined with chemotherapy. Predictive biomarkers were identified using tissue microarrays and immunohistochemistry (IHC) examining a variety of molecules, including expression of ErbB receptors and their activation/phosphorylation state, as well as ErbB receptor ligands and downstream mediators of proliferation and survival signals (Figures 6 & 7) [114]. The results of this analysis identified a set of biomarkers that best predict patient outcome following trastuzumab combination therapies. Patient probability of response ranging from 0 to 100% was observed based upon the expression or phosphorylation of a small set of growth factor receptors, downstream signaling proteins, and ErbB receptor ligands (Table 3).

The success of trastuzumab therapy in the treatment of breast cancer patients has been limited, despite selecting for patients whose tumors overexpress the ErbB2 protein. Our results demonstrated that the status of IGF-IR and the ErbB receptor ligands, HRG and TGFα, affect the response to trastuzumab therapy in breast cancer patients. Patients whose tumors express high levels of ErbB1 and B2 and HRG or TGFα are most likely to respond to trastuzumab. Other studies in cell lines have shown that not all tumor cells respond to inhibition of ErbB receptors, despite exhibiting aberrant ErbB1 and/or ErbB2 expression. In this respect, it has been reported that a combination of the ErbB1-directed mAb C225 and the ErbB2-directed mAb 4D5, or use of the dual ErbB1/B2 small molecule tyrosine kinase inhibitors, provides a more potent antitumor effect compared with using either mAb alone. A diagnostic protocol for predicting patient response to trastuzumab and chemotherapy is presented (Figure 8). The left arm of the protocol details the analysis of the targeted pathways, namely the ErbB receptors and their ligands. The right arm of the protocol details the analysis of alternative pathways, namely the IGF-IR pathway and downstream signaling which is associated with resistance to trastuzumab.

Our results suggest that the IGF-IR receptor may influence patient response to breast cancer therapies targeting ErbB2. High IGF-IR
expression combined with high S6 ribosomal protein phosphorylation correlated with poor patient response to herceptin regardless of ErbB expression, indicating that IGF-IR was directly activating downstream signaling pathways rather than exerting its effects through transactivation of ErbB receptors. IGF signaling in breast cancer has been shown to occur through AKT activation [114,127], which would lead to S6 ribosomal protein phosphorylation. Hence, S6 phosphorylation may indicate active IGF signaling in those tumors overexpressing IGF-IR [127]. Based upon our results, as well as the results of these published studies, analysis of IGR-IR expression and downstream signaling may be critical for an accurate assessment of potential trastuzumab response in breast cancer patients (right arm of Figure 8). In addition, activation of phosphatase and tensin homolog deleted on chromosome ten (PTEN) – a dual phosphatase that dephosphorylates P13K/Akt – was shown to be part of the mechanism involved in trastuzumab response. Trastuzumab increased PTEN membrane localization, increased its phosphatase activity and reduced its phosphorylation by reducing SRC and ErbB2 association. Patients who were PTEN deficient had a poorer response to trastuzumab [128].

**Antibodies for ErbB & B2 in combination with chemotherapy**

Cisplatin (CDDP) is a DNA-damaging antitumor agent employed for the treatment of various human cancers. CDDP activates nuclear as well as cytoplasmatic signaling pathways involved in regulation of the cell cycle, damage repair and programmed cell death. ErbB1/EGFR is activated in response to CDDP in various types of cells that overexpress the receptor, including transformed human glioma cells and human breast tumor cells. Activation of ErbB1 in response to CDDP requires its kinase activity, as it can be blocked by an ErbB1 kinase inhibitor or expression of a kinase dead receptor. CDDP-induced ErbB1 activation is independent of the receptor ligand. CDDP induces the activation of c-Src and ErbB1, both of which are blocked by PP1, a Src inhibitor, suggesting that Src kinases mediate CDDP-induced ErbB1 activation. ErbB1 activation in response to CDDP is a survival

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**Table 1. Monoclonal antibodies designed to target the ErbB family.**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Characteristic</th>
<th>Target</th>
<th>Tumor type</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab</td>
<td>Chimeric</td>
<td>ErbB1</td>
<td>Colon, H&amp;N, NSCLC and pancreas</td>
<td>Marketed</td>
</tr>
<tr>
<td>Panitumab</td>
<td>Human</td>
<td>ErbB1</td>
<td>Colon, renal</td>
<td>Phase III</td>
</tr>
<tr>
<td>Matuzumab</td>
<td>Humanized</td>
<td>ErbB1</td>
<td>H&amp;N, ovarian, colon and cervix</td>
<td>Phase III</td>
</tr>
<tr>
<td>h-R3</td>
<td>Humanized</td>
<td>ErbB1</td>
<td>H&amp;N</td>
<td>Phase II</td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>Humanized</td>
<td>ErbB2</td>
<td>Breast, ovarian, prostate and NSCLC</td>
<td>Phase II</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Humanized</td>
<td>ErbB2</td>
<td>Breast</td>
<td>Marketed</td>
</tr>
</tbody>
</table>

H&N: Head and neck; NSCLC: Non-small cell lung cancer.

**Table 2. Tyrosine kinase inhibitors designed to target the ErbB family.**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Irreversible?</th>
<th>Target</th>
<th>Tumor type</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td>No</td>
<td>ErbB1</td>
<td>NSCLC</td>
<td>Marketed</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>No</td>
<td>ErbB1</td>
<td>NSCLC, pancreas</td>
<td>Marketed</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>No</td>
<td>ErbB1/2</td>
<td>Breast</td>
<td>Phase III</td>
</tr>
<tr>
<td>CI-1033</td>
<td>Yes</td>
<td>Pan ErbB</td>
<td>SCC, skin</td>
<td>Phase II</td>
</tr>
<tr>
<td>EKB-569</td>
<td>Yes</td>
<td>ErbB1</td>
<td>Colon</td>
<td>Phase II</td>
</tr>
<tr>
<td>BMS-599626</td>
<td>No</td>
<td>ErbB1/2</td>
<td>Colon</td>
<td>Phase I</td>
</tr>
<tr>
<td>AEE788</td>
<td>No</td>
<td>ErbB1/2</td>
<td>Anti-VEGFR</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; VEGFR: Vascular endothelial growth factor receptor.
response, since inhibition of ErbB1 activation enhances CDDP-induced death. These findings demonstrate that signals generated by DNA damage can modulate ErbB1 activity, and argue that interfering with CDDP-induced ErbB1 activation in tumor cells might be a useful approach to sensitize these cells to genotoxic agents [123].

Trastuzumab increases the clinical benefit of first-line chemotherapy in patients with metastatic breast cancers that overexpress ErbB2 (HER2). Additive interactions were observed in four tumor cell lines treated with trastuzumab in combination with doxorubicin, epirubicin and paclitaxel. Interactions between trastuzumab and gemcitabine were synergistic at low concentrations of gemcitabine and antagonistic at high concentrations. A synergistic interaction was observed with a three-drug combination of docetaxel/carboplatin/trastuzumab in SKBR3 breast cancer cells. Consistent synergistic interactions of trastuzumab and carboplatin, 4-hydroxycyclophosphamide, docetaxel, or vinorelbine across a wide range of clinically relevant concentrations in ErbB2-overexpressing breast cancer cells indicate that these are rational combinations to test in clinical trials [91].

**Tyrosine kinase inhibitors to ErbB1 & B2**

This class of agents competes with adenosine triphosphate (ATP) binding to the TK domain of the receptor, leading to inhibition of TK activity and subsequent abrogation of ErbB receptor signaling (Figure 9). A significant number of these small molecule ErbB TK inhibitors are currently under clinical development (Table 2). They differ mainly in their potency against the different members of the ErbB receptor family and their capacity to:

- Preferentially inhibit a single receptor type
- Inhibit multiple ErbB receptors
- Inhibit members of other TK-receptor families

Based on the early clinical activity observed in the Phase I studies, anti-ErbB1 TK inhibitors were preferentially studied in patients with advanced NSCLC [14,15]. Erlotinib (Tarceva®, Roche) is an orally bioavailable ErbB1 tyrosine kinase inhibitor that has been evaluated for the treatment of a range of human epithelial malignancies. Erlotinib and radiation induce the accumulation of tumor cells in the G1 and G2-M phases of the cell cycle, respectively, with a reduction of cells in S phase. When combined, erlotinib and radiation promote a further reduction in the S-phase fraction. By inhibiting ErbB1 autophosphorylation, erlotinib appears to enhance tumor cell apoptosis, promoting sensitization to radiation therapy. Preliminary microarray data suggest additional mechanisms underlying the complex interaction between ErbB1 signaling and the response to radiation therapy. These data suggest that the erlotinib/radiation combination represents a strategy worthy of further examination in clinical trials.

### Table 3. Biomarkers that predict response to trastuzumab.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Nonprogressors (%)</th>
<th>Progressors (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ErbB1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>positive</td>
<td>43</td>
<td>30</td>
<td>70</td>
<td>0.05</td>
</tr>
<tr>
<td>negative</td>
<td>23</td>
<td>9</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>66</td>
<td>23</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td><strong>NDF/Heregulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>55</td>
<td>39</td>
<td>61</td>
<td>0.02</td>
</tr>
<tr>
<td>negative</td>
<td>22</td>
<td>9</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>77</td>
<td>30</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td><strong>IGF-1R/pS6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pos/pos</td>
<td>13</td>
<td>8</td>
<td>92</td>
<td>0.02</td>
</tr>
<tr>
<td>neg/pos</td>
<td>12</td>
<td>67</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td><strong>NDF/pS6/IGF-1R</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+/#</td>
<td>4</td>
<td>0</td>
<td>100</td>
<td>0.01</td>
</tr>
<tr>
<td>+//-</td>
<td>7</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from [114]. IGF-IR: insulin-like growth factor receptor; NDF: Neu differentiation factor.
responsiveness to the tyrosine kinase inhibitor gefitinib. These mutations lead to increased growth factor signaling and confer susceptibility to the inhibitor. Somatic mutations were identified in the tyrosine kinase domain of the EGFR gene in eight out of nine patients with gefitinib-responsive lung cancer, in contrast to none of the seven patients with no response. Mutations were either small in-frame deletions, or amino acid substitutions clustered around the ATP-binding pocket of the tyrosine kinase domain. Similar mutations were detected in tumors from patients with primary NSCLC who had not been exposed to gefitinib (8%). All mutations were heterozygous, and identical mutations were observed in multiple patients, suggesting an additive specific gain of function. In vitro, EGFR mutants demonstrated enhanced tyrosine kinase activity in response to EGF and increased sensitivity to inhibition by gefitinib. Screening for such mutations in lung cancers may identify patients who are more likely to respond to gefitinib.

Lapatinib (GW572016) is an orally-active small molecule that reversibly inhibits ErbB1 and B2 tyrosine kinases, which in turn blocks phosphorylation and activation of Erk1/2 (p-Erk1/2) and Akt (p-Akt) in ErbB1- and/or ErbB2-expressing tumor cell lines and xenografts. Lapatinib elicits cytostatic or cytotoxic antitumor effects depending on the cell type. Since ErbB2-containing heterodimers exert potent mitogenic signals, simultaneously interrupting both ErbB1 and B2 signaling is an appealing therapeutic approach. Our laboratories conducted various studies to understand the molecular factors associated with response to ErbB1/B2-targeted therapies and to elucidate the biologic pathway of lapatinib resistance on breast cancers and their correlation with clinical response and treatments.

In a Phase Ib study, responders to lapatinib exhibited variable levels of inhibition of p-ErbB1, p-ErbB2, p-Erk1/2, p-Akt, cyclin D1, and TGFα. Even some nonresponders demonstrated varying degrees of biomarker inhibition. Increased tumor cell apoptosis (detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling [TUNEL]) occurred in patients with evidence of tumor regression but not in nonresponders (progressive disease). Clinical response was associated with a pretreatment TUNEL score greater than 0 and increased pretreatment expression of ErbB2, p-ErbB2, Erk1/2, p-Erk1/2, IGF-IR, p70S6K, and TGFα compared with nonresponders. In addition, expression of the estrogen receptor (determined by archived tissue) appears to be associated with a decreased response rate to lapatinib monotherapy in patients with ErbB2-overexpressing tumors (determined by archived tissue) who had progressive disease despite prior trastuzumab therapy. However, this association will
require confirmation in larger series of patients. Below is a summary of the factors that, at this time, appear to be the best candidates to predict response (or conversely resistance) to lapatinib monotherapy:

- TUNEL
- ErbB2
- p-ErbB2
- TGFα
- p-ERK1/2
- ER
- PR

From the bench to the bedside

The multiple tests that are needed for breast cancer patients before therapeutic intervention are depicted in Figure 10, and the advances for personalized medicine are elucidated. Patient’s breast cancer tissue were stained by IHC for various factors associated with breasts targeted for therapy.

The patient exhibits infiltrating ductal carcinoma (hematoxylin and eosin). Fluorescent in situ hybridization analysis demonstrates ErbB2 amplification, and IHC shows overexpression of ErbB2 and expression of ER and PR. Targeted therapy for ErbB alone may not be optional as the cancer expresses ERs. This patient will probably benefit from a combination of ErbB2 inhibitors together with antihormone therapy [128]. In ongoing clinical studies in the authors’ laboratory, other laboratories [130], and in a paper published by the authors’ group [129], the approach was to use biomarkers to stratify breast cancer patients in early clinical trials that were treated with lapatinib in combination with chemotherapy and antihormonal therapy. Larger clinical trials using this approach are needed.

**Major messages**

Targeted cancer therapies intercept specific steps in signal transduction and accelerate target degradation. Patient response to drugs (e.g., trastuzumab and gefitinib) correlates with the presence of genetic aberrations.

It is important to use biomarkers for patients undergoing a particular treatment or therapy, or who have previously undergone a particular treatment or therapy. It can be used before any treatment is initiated or prior to use of any therapeutic agent. Thus, use of the targeted therapy markers allows earlier identification of treatments and therapeutic agents likely to result in a clinically positive outcome. In addition, use of the targeted therapy markers allows earlier identification of treatments and therapeutic agents that are not likely to result in a clinically positive outcome.

**Conclusion**

To elucidate the best patient population for a specific targeted therapy alone or in combination, one needs to understand the drug’s activity. Advances toward personalized medicine for cancer patients, with the advent of genetic understanding of cancer treatments, are going to integrate the specific molecular alternatives in each patient’s cancer to optimize patient’s responses.
ErbB receptor targeted therapies – REVIEW

Figure 10. Testing of breast cancer tissue from markers associated with response to ErbB inhibitors and chemotherapeutic agent immunohistochemistry and fluorescent in situ hybridization (40×).

ER: Estrogen receptor; FISH: Fluorescent in situ hybridization; H&E: Hematoxylin and eosin; HER: Human epidermal growth factor receptor; IHC: Immunohistochemistry; PR: Progesterone receptor.

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**The role of ErbB2 in breast cancer clinical behaviors.**


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