Clinical activities of the epidermal growth factor receptor family inhibitors in breast cancer

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Abstract: The epidermal growth factor (EGF) receptors play an important role in epithelial cell function. Upon stimulation of these receptors, an extensive network of signal transduction pathways is activated, including the PI3K/AKT and Ras/Erk pathways. This activation leads to cellular proliferation and survival. In breast cancer, the EGF receptor, ErbB2 (HER2/neu), can be amplified and over-expressed and this is associated with poor prognosis and drug resistance. Trastuzumab is a monoclonal antibody against ErbB2 and has demonstrated activity in the therapy of breast cancer patients with over-expression of ErbB2, both in the metastatic and adjuvant setting. Recently, a tyrosine kinase inhibitor, lapatinib, that targets both ErbB1 and ErbB2, has also shown activity in metastatic breast cancer. In this review, we will discuss the ErbB receptors and their signaling networks in breast cancer, as well as the clinical activities of trastuzumab and lapatinib in this disease.

Keywords: trastuzumab, lapatinib, ErbB receptors, breast cancer and tyrosine kinases

Introduction

Some of the most important signaling molecules that maintain cell survival and proliferation are the growth factor receptors. Epidermal growth factor (EGF) receptors are key regulatory factors in promoting both cell survival and proliferation in epithelial cells. EGF receptors (ErbB receptor family) transduces specific cellular signals to the cell leading to specific cellular responses through their intracellular tyrosine kinase domain (Burgess et al 2003; Herbst 2004). In breast cancer, these ErbB receptors are up-regulated, their tyrosine kinase activity is increased and their signaling pathways are constitutively activated making inhibiting ErbB receptor tyrosine kinase activity an attractive target in this disease (Hynes and Lane 2005). The development of antagonists to one of these ErbB receptors, ErbB2, is an example of how molecular-targeted therapy is utilized in breast cancer therapy (Nahta and Esteva 2007). In this review, we will discuss how ErbB receptors are activated and how using ErbB receptor inhibitors are being developed as effective treatments for breast cancer.

EGF family of receptors (Figure 1)

The EGF receptor (ErbB) family consists of four closely related tyrosine kinase transmembrane receptors: ErbB1 (EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) that bind to an array of ligands (Normanno et al 2005). These ligands can be classified in two categories, ligands that predominantly bind to only ErbB1 and ligands that bind to ErbB3 and/or ErbB4. EGF binds to only ErbB1 whereas other ligands such as neuregulin bind to ErbB3 and ErbB4. Upon binding to their corresponding ligands, the ErbB receptors form homo- or hetero-dimers. Upon EGF ligation with ErbB1, the receptor either homo-dimerizes or hetero-dimerizes with the other three ErbB receptors. ErbB2 fails to bind to ErbB receptor ligands, indicating ErbB2 is a coreceptor contributing to the activation of the other ErbB receptors through dimerization. ErbB3 has an inactive kinase domain which indicates that is also acts as a co-receptor for activation of other ErbB receptor members

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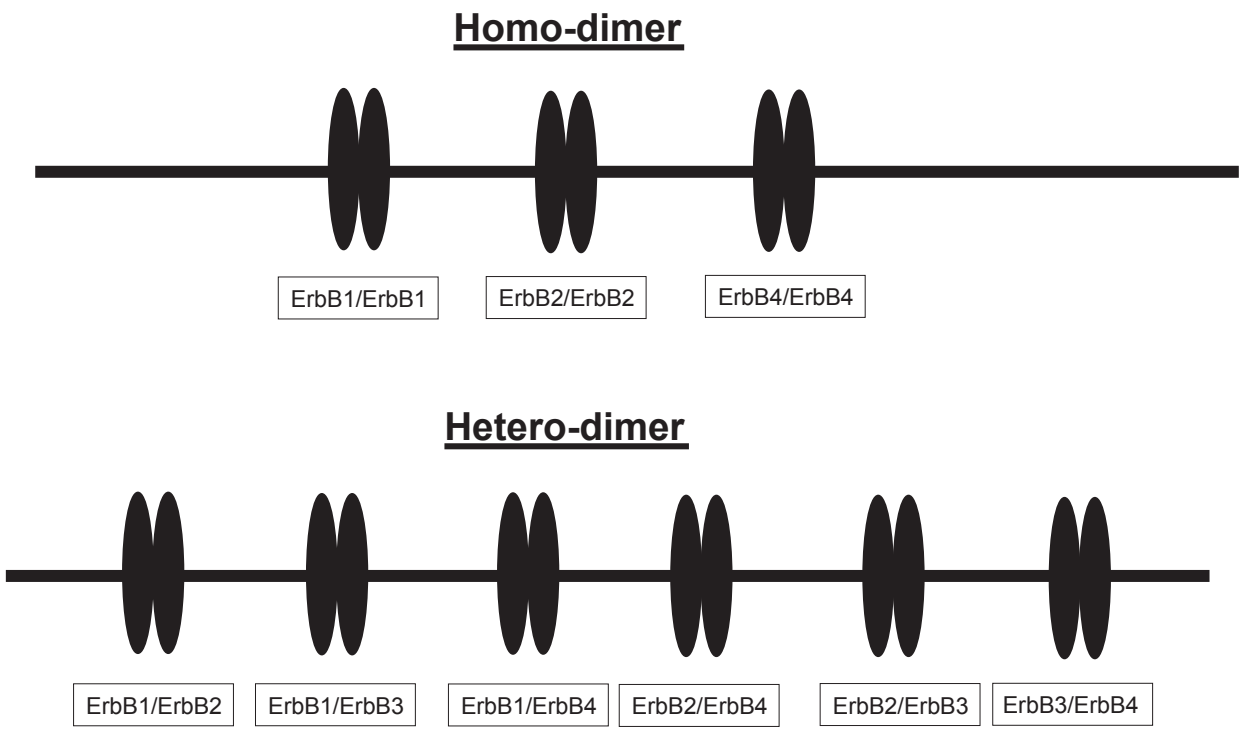

Figure 1 ErbB receptors form homo- and hetero-dimers upon activation. The ErbB receptor family consists of ErbB1 (EGFR), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). These receptors form homo- or hetero-dimers upon activation. The only exception is ErbB3 homo-dimer since ErbB3 has a non-functional tyrosine kinase domain. ErbB2 homo-dimers occur independent of ligand whereas the other dimers depend on binding of ligands for activation.

(Normanno et al 2005). After dimerization, the tyrosine kinase domain in the receptors phosphorylates tyrosine residues in the neighboring receptor. This allows recruitment of adaptor proteins, such as Grb2, to receptors and the initiation of signal transduction pathways. The receptors are then often endocytozed and bind to an adaptor protein, c-cbl, that targets ErbB receptors for degradation (Muthuswamy et al 1999). Other receptors are recycled back to the plasma membrane where they are available to bind to other ligands (Burgess et al 2003).

ErbB receptors are expressed in a variety of human tissues of epithelial, mesenchymal and neuronal origin. The biological function of these receptors has been demonstrated in a number of different mouse models. Mice lacking ErbB1 have abnormal eyes and epidermal tissues and die due to defects in epithelial organ development. In mice lacking ErbB2, a malformation of the heart is found and this contributes to death of the mice at midgestation. ErbB3 knockout mice die from defects in the heart and neural crest, and lack Schwann cell precursors (Olayioye et al 2000).

ErbB receptor signaling pathways (Figure 2)

Upon ligation, ErbB receptors are activated. The activated receptor kinase phosphorylates tyrosine residues on the C-terminal tail of the ErbB receptors (Muthuswamy et al 1999; Belsches-Jablonski et al 2001). These tyrosine phosphorylated sites allow the binding of proteins containing the Src Homology 2 (SH2) domain. These proteins consist of intracellular docking proteins or adaptor proteins, such as Grb2 and Shc. Upon binding to the ErbB receptors, these protein associate with other proteins leading to the activation of serine threonine kinases that phosphorylate serine or threonine residues on other protein kinases and/or transcription factors (Olayioye et al 2000). This kinase cascade leads to amplification of a network of signaling pathways resulting in changes in protein functions and activation of gene transcription. Two of the most prominent signaling pathways found after ErbB receptor activation are the Ras/Erk and the PI3K/AKT pathways. Mitogen activated protein kinases (MAPKs) are a superfamily of protein serine-threonine kinases in which Erk1/2 are members. ErbB receptors activate Erks through the binding of adaptor protein Grb2 to ErbB receptor recruiting son of sevenless (SOS) protein to the receptor (Wu et al 1993). SOS is a guanyl nucleotide-release protein (GNRP) that upon recruitment to the plasma membrane by the activated cell surface receptor causes the small G protein RAS to release GDP and exchange it for GTP. When Ras has GTP bound to it, it becomes active. Activation of Ras

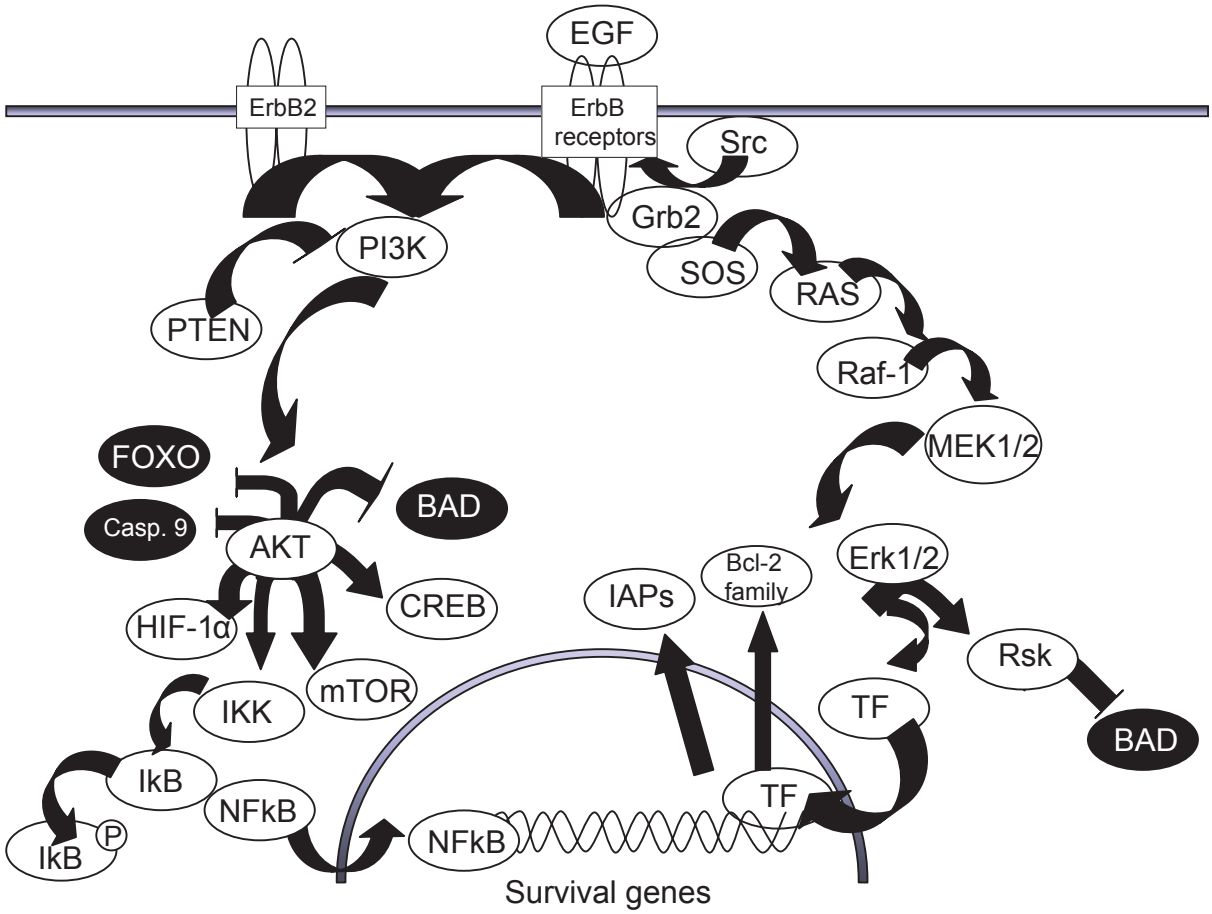

Figure 2 ErbB receptor signaling. EGF binds to its ErbB receptors leading to activation of a network of signaling pathways, including the PI3K/AKT and RAS/ERK pathways. These pathways lead to the activation of anti-apoptotic proteins (white) and the inactivation of pro-apoptotic proteins (black). ErbB2 homodimers activate the same pathways but do not require EGF for activation and ErbB receptors can also be activated through tyrosine phosphorylation of the ErbB receptors mediated by non-tyrosine kinase Src.

leads to the activation of the MKKK Raf-1, Raf-1 kinase then phosphorylates and activates the MKK Mek1/2. Activated Mek1/2 then phosphorylates and activates Erk1/2 (Johnson et al 2005). This increased expression results in phosphorylation of a variety of substrates including 90 kDa ribosomal S6 protein kinase (Rsk), Msk1, cytosolic phospholipase A2, and transcription factors c-Myc, NF-IL6, Tal-1, Ets-2, and Elk (Widmann et al 1999). As a result, there is enhanced gene transcription with increased cell survival and proliferation (Lin et al 2002).

ErbB receptors also activate the PI3K/AKT pathway through recruitment of PI3K to the plasma membrane and this is mediated by binding of its SH2 domain to tyrosine phosphorylated proteins. PI3K activation catalyzes the transfer of a phosphate group from ATP to phosphatidylinositol generating a 3'-phosphatidylinositol phosphate (PIP). PIPs act as binding sites for proteins with pleckstrin homology (PH) domains. PI3K signaling is negatively regulated by PTEN that dephosphorylates PIPs. AKT is a serine/threonine kinase that contains a PH domain. AKT's PH domain binds to phosphatidylinositols generated by PI3K translocating AKT to the plasma membrane where it becomes phosphorylated and activated (Song et al 2005). Activated AKT can either activate or inhibit a number of downstream targets that participate in cellular survival. AKT activates transcription factor NFκB, HIF-1α and CREB resulting in increased transcription of anti-apoptotic genes such as growth factors and Bcl-2 family members that prevent cell death. In contrast, AKT inactivates transcription factors Foxo (forkhead family) and p53, either directly phosphorylating Foxo proteins or by phosphorylating and activating Mdm2, a negative regulator of p53 (Brunet et al 1999; Zhou et al 2001). In both cases, pro-apoptotic gene expression is decreased and cell survival is increased. AKT also phosphorylates and activates mTOR, a serine/threonine kinase. mTOR activation lead to phosphorylation and activation of ribosomal S6 kinase and the eukaryotic initiation factor 4E (eIF4E) – binding protein 1 (4E-BP1). This increases the translational capacity within

cells and has been implicated in cell survival (Chan 2004; Sun et al 2005).

ErbB receptor family and breast cancer

ErbB receptor family members were first implicated in cancer in the early 1980s when the avian erythroblastosis tumor virus was found to encode an aberrant form of the receptor ErbB1 (Herbst 2004). Since then, activation of ErbB receptor family members has been implicated in the pathogenesis of gliomas and lung, head and neck and renal carcinomas. However, it is the activation of ErbB receptors in breast cancer that has been the focus of the most research and clinical investigation (Janmaat and Giaccone 2003). In breast cancer, ErbB2 expression is increased in approximately 25% of tumors, as a result of gene amplification (Normanno et al 2005). Since ErbB2 does not require a ligand to become activated, increased expression in breast cancer causes increased ErbB2 signaling through homo- and hetero-dimerization and this leads to to increased resistance to cell death (apoptosis), increased cell proliferation and enhanced invasiveness. Breast cancer patients with with high ErbB2 expression generally have aggressive disease and poor prognosis (Normanno et al 2005). In addition, ErbB1 expression is elevated in about 30% of breast tumors and is often complexed with ErbB2. The ErbB1 gene is rarely mutated in breast cancer but in lung cancer and glioblastoma multiforme, the intracellular domain of ErbB1 is mutated rendering the tyrosine kinase constitutively active (Pedersen et al 2001). These patients generally have aggressive disease. The microenvironment in breast cancer can also influence ErbB receptor signaling as the stromal cells may secrete EGF which then leads to activation of the receptors (Normanno et al 2005).

Activation of the Ras/Erk signaling pathway by ErbB is deregulated by mutations in Ras that increase Erk signaling (Rajagopalan et al 2002; Duursma and Agami 2003). Many breast cancers also contain mutations in the tumor suppressor PTEN, that dephosphoryates the second messenger lipid, PtdIns (3,4,5) triphosphate, causing down-regulation of the PI3K/AKT signaling pathway. In approximately 25% of breast tumors, PTEN is mutated and this inhibits its lipid phosphatase activity which increases EGF-mediated AKT activation and cell survival (Sansal and Sellers 2004). In addition, transcription factors are often altered in breast cancer. NFκB and CREB transcriptional activation are often increased whereas tumor suppressor p53 is mutated in many breast cancers (Dolcet et al 2005; Shankar et al 2005; Yu and Zhang 2005). As a result, there is an alteration in the expression of the pro- and anti-apoptotic proteins. For example, anti-apoptotic Bcl-2 family members Bcl-2, Bcl-x_L and Mcl-1 levels are often elevated in breast cancer and this is associated with drug resistance (Kumar et al 1996; Stoll et al 1998; Wang et al 1999; Henson et al 2003; Yu and Zhang 2005). The multi-faceted alterations in the ErbB receptor mediated signaling pathways in breast cancer ensure that the cells can survive in different environments and contribute to tumor progression. As a result, these receptors, especially ErbB2, are attractive targets for cancer therapy.

ErbB receptor inhibitors in breast cancer therapy (Table 1)

There are two classes of ErbB receptor inhibitors. The first class of inhibitors are monoclonal antibodies which are directed against the ErbB receptors and these inhibit ErbB signaling and may induce immune mediated cell killing. These monoclonals target the over-expression of ErbB in breast cancer and the most effective antibody to date is trastuzumab (Dassonville et al 2007; Nahta and Esteva 2007). The second class of inhibitors are tyrosine kinase inhibitors that inhibit the tyrosine kinase activity of ErbB receptors and thereby inhibit ErbB receptor signaling. The most promising tyrosine kinase inhibitor in breast cancer treatment is lapatinib that blocks the activation of ErbB1 and ErbB2. Lapatinib has similar effects to trastuzumab and may be particularly effective when combined with trastuzumab (Moy and Goss 2006). The clinical responses for transtuzumab and lapatinib in breast cancer are discussed below.

Trastuzumab in breast cancer

Trastuzumab is the first monoclonal antibody targeting ErbB2 that has been approved for clinical use. This antibody binds to ErbB2, causing receptor endocytosis and/or ablation of ErbB2 signaling, which include the activation of the Ras/Erk and PI3K/AKT pathways (Johnston et al 2006). In addition, trastuzumab contains a human IgG_1Fc region that activates antibody-dependent cellular cytotoxicity (ADCC) when it binds to ErbB2 (Hynes and Lane 2005). The initial clinical trials with trastuzumab in metastatic breast cancer over-expressing ErbB2 showed response rates ranging from 12 to 34% (Baselga et al 1996; Cobleigh et al 1999; Vogel et al 2002). Subsequent studies combined trastuzumab with paclitaxel or docetaxel in metastatic breast cancer. Response rates ranged from 50% to 72%, with the time to progression ranging from 7.4 to 9 months, and overall survival from 25 to 31 months (Seidman et al 2001; Slamon et al 2001; Esteva et al 2002). These results were significantly higher than

Table 1 Clinical trials for trastuzumab and lapatinib

Treatment	Phase	Patient number	Combination	Tumor type	Dose	Complete response	Partial response	Stable disease	Time to progression	Median survival	Ref.
Trastuzumab	II	213	Single agent	ErbB2 overexpressing metastatic breast cancer with progressive disease	Loading dose: 4 mg/kg with weekly administration of 2-mg/kg	4%	12%		9.1 months	13 months	Cobleigh1999
	III	64	Chemotherapy alone (n = 19) or in combination (n = 45) with trastuzumab (3)	ErbB2 positive, non-inflammatory breast cancer.	Paclitaxel 225 mg/m^2, fluorouracil, 500 mg/m^2, cyclophosphamide 500 mg/m^2, and 75 mg/m^2 epirubicin. trastuzumab loading dose: 4 mg/kg with weekly administration of 2 mg/kg	P+FEC 54.5% P+FEC+H 60%				P+FEC 85.3% P+FEC+H 100% 36.1 months	Buzdar 2007
Lapatinib	I	67 (30)	Single agent	Heavily pretreated ErbB1 and/or ErbB2 positive metastatic cancer	Dose range of 500, 650, 900, 1200 and 1600 mg of lapatinib		10%	10%			Burris 2005(1)
	II	39	Single agent	ErbB2 positive breast cancer with new or progressing brain metastases	750 mg lapatinib twice daily		5% had CNS partial response, 25% had non-CNS partial response	20% had stable disease in CNS at 16 weeks	3.02 months	6.57 months	Lin 2006
	III	324	Capecitabine alone (n = 161) or in combination with Labatinib (n = 163)	ErbB2 positive breast cancer that progressed after treatment (2).	1250 mg lapatinib daily with capecitabine 2000 mg/m^2 two divided doses days 1–14 of a 21-day cycle. Single dose capecitabine administered at 2500 mg/m^2 two divided doses days 1–14 of a 21-day cycle.	Capecitabine alone (C) 0% Capecitabine and lapatinib (C+L) <1%	C = 14% CL = 22%	C = 18% CL = 27%	C = 4.4 months CL = 8.4 months		Geyer 2006

(*Continued*)

Table 1 (*Continued*)

Treatment	Phase	Patient number	Combination	Tumor type	Dose	Complete response	Partial response	Stable disease	Time to progression	Median survival	Ref.
	III		Currently enrolling patients. This study is designed for 3000 patients ErbB2 positive breast cancer, who have completed adjuvant therapy. Patients will receive single agent lapatinib or placebo for one year.								TEACH trial
	III		Currently enrolling patients. This study is designed for 1200 patients who are post-menopausal, with ER positive metastatic breast cancer. Patients will receive the aromatase inhibitor letrozole (2.5 mg) combined with either labatinib (1500 mg) or a placebo.								EGF 30008
Traztuzumab and lapatinib	I	27	In combination	ErbB2 positive breast cancer	Lapatinib 750–1500 mg, trastuzumab loading dose: 4 mg/kg with weekly administration of 2 mg/kg	3.7%	18.5%	37%			Storniolo 2005 ASCO meeting
	III		Currently enrolling patients. This study is designed for 8000 patients with early stage ErbB2 positive breast cancer. There will be four treatment arms: trastuzumab alone, lapatinib alone, trastuzumab followed by lapatinib and finally trastuzumab plus lapatinib.								ALTO trial

(1) Four advanced stage patients that had progressed on either trastuzumab and taxane treatment or trastuzumab and anthracycline regimes achieved a partial response with lapatinib.
(2) Patients progressed on treatment that included an anthracycline, a taxane and trastuzumab.
(3) Patients received either four cycles of paclitaxel followed by four cycles of FEC (concurrent 5-fluorouracil, epirubicin, and cyclophosphamide) or trastuzumab plus four cycles of paclitaxel followed by four cycles of FEC (P+FEC or P+FEC+H).

when patients were treated with taxenes alone. In addition, in patients with metastastic breast cancer with amplification of the ErbB2 gene, there was a median time to progression of 4.9 months with trastuzumab mono-therapy compared to 7.4 months when trastuzumab was combined with chemotherapy, such as paclitaxel (Slamon et al 2001). When used in the adjuvant setting in breast cancer, trastuzumab was found to improve disease-free and overall survival rates when given in combination with chemotherapy or following chemotherapy (Piccart-Gebhart et al 2005; Romond et al 2005). In one study, patients were given doxorubicin/cyclophosphamide followed by 12-weekly doses of paclitaxel (group A) while the second group (group B) was given doxorubicin/cyclophosphamide followed by 52 weeks of trastuzumab and paclitaxel. The result was an increase in time to progression in group B from 67 months to 87 months and increased overall survival group B from 86% to 91% (Romond et al 2005). This led to the approval of trastuzumab as a therapy in early stage breast cancer patients with elevated ErbB2 expression.

Lapatinib therapy in breast cancer

The second class of ErbB receptor inhibitors are the tyrosine kinase inhibitors. This type of inhibitor provides an advantage over monoclonal antibodies as it directly inhibits ErbB receptor signaling by blocking the receptor's kinase activity. Tyrosine kinase inhibitors are also taken orally as opposed to monoclonal antibodies which need to be administered intravenously. This eliminates the need for patients to attend the chemotherapy unit for treatments and the risk of allergic reactions to the antibody (Moy and Goss 2006).

The first ErbB tyrosine kinase inhibitors, gefitinib and erlotinib, were against ErbB1. These inhibitors are effective against non-small cell lung cancers that over express ErbB1, and are particularly effective against cells expressing a mutation in the kinase domain that renders these receptors constitutively active (Johnston et al 2006). Lapatinib is a tyrosine kinase inhibitor that inhibits the kinase activity of ErbB1 and ErbB2. In preclinical studies, lapatinib inhibited ErbB1 and ErbB2 phosphorylation in an ErbB2 over-expressing BT474 breast cancer cell line. In mouse xenografts, the growth of BT474 tumors was inhibited by 94% following treatment with lapatinib. The activation of Erk and AKT was also inhibited (Xia et al 2004). These results suggest that Erk and/or AKT activation could be a useful biomarker for responsiveness to tyrosine kinase inhibitors. Preclinical studies have also demonstrated that lapatinib can block growth in breast cancer cell lines. Lapatinib caused 50% reduction in kinase activity of ErbB1 and ErbB2 at concentrations less than 0.2 μM in several cell lines and xenograft tumor

model (Xia et al 2002). Lapatinib is also effective in breast cancer cell lines that are resistant to trastuzumab treatment (Xia et al 2004; Zhou et al 2004). There is a significant correlation with response to lapatinib and ErbB2 expression and its ability to inhibit Raf, Erk and AKT activation (Konecny et al 2006). In murine mammary xenografts of estrogen receptor positive, tamoxifen resistant tumors, lapatinib restored tamoxifen sensitivity. ErbB receptors have been implicated in endocrine resistance in breast cancer cells through phosphorylation of the estrogen receptor by Erk and AKT signaling pathways (Konecny et al 2006). Lapatinib could reverse this resistance indicating that combining tamoxifen with lapatinib might be a rational and effective treatment. Indeed, a combination of lapatinib and tamoxifen produced a greater antiproliferative effect in breast cancer cells than either drug alone (Chu et al 2005). Furthermore, trastuzumab in combination with lapatinib showed a synergistic effect on the proliferative capacity of several breast cancer cell lines (Konecny et al 2006). Finally, through effects on ErbB signaling, lapatinib has been shown to sensitize breast cancer cells to irradiation in vitro (Zhou et al 2004). These preclinical results provide the rationale to develop clinical trials in breast cancer using lapatinib in combination with other treatment modalities.

A phase I clinical trial on 67 previously treated breast cancer patients with overexpression of ErbB1 and ErbB2 and metastatic disease showed that lapatinib was well tolerated, and side-effects included diarrhea, rash and fatigue, which are typically seen with ErbB targeted therapies. Importantly, none of the patients experienced cardiac toxicity (Moy and Goss 2006; Ito et al 2007). Phase II clinical trials are ongoing to evaluate lapatinib activity in metastatic breast cancer which is refractory to trastuzumab, anthracyclines, taxenes, and capecitabine. Interim analysis of one these trials revealed two partial responses and 17 stable disease (Geyer et al 2006). When lapatinib was used alone in trastuzumab naïve breast cancer patients, the response rate was 24% and the median time to treatment failure was 16.1 weeks (Ito et al 2007). In addition, inflammatory breast cancer patients that were ErbB2 over-expressing showed a 72% response to lapatinib alone (Tuma 2007). Lapatinib has also been studies in combination with cytotoxic drugs. In a randomized phase III trial, 528 patients with ErbB2-expressing breast tumors who had been previously been treated with anthracyclines, taxenes or trastuzumab received either capecitabine alone or capecitabine in combination with lapatinib (Geyer et al 2006). Of the 324 patients initially analyzed, the combination treatment showed a 22% response rate while capecitabine alone gave a 14% response rate. The time to progression was 8.4 months with the combination treatment and 4.4 months with capecitabine alone ($p < 0.001$, hazard ratio = 0.47). Of note, there was a reduced incidence of metastasatic disease to the central nervous system in patients treated with the combination treatment and no increase in toxicity (Geyer et al 2006). This has led the Food and Drug Administration (FDA) to approve the use of the lapatinib/capecitabine combination in metastatic breast cancer (Engel and Kaklamani 2007). Other phase III clinical trials are ongoing comparing lapatinib and paclitaxel as first line therapies for ErbB2 over-expressing locally advanced or metastatic breast cancer. The preliminary results show that 77% of patients have a clinical response and pathological complete response was observed in 17% of patients with inflammatory breast cancer following lapatinib treatment. However, the number of patients are relatively small (30 patients treated) (Ito et al 2007).

These results indicate that lapatinib might be as effective as trastuzumab in ErbB2-expressing breast cancers and leads to the question as to whether combining trastuzumab and lapatinib might increase the response rate and survival. In support of this concept are the results of preclinical investigations which indicate that a synergistic antitumor effect is seen in ErbB2-positive breast cancer cells treated with a combination of trastuzumab and lapatinib, and this effect is associated with a decrease in the expression of the anti-apoptotic protein, survivin (Xia, Bisi et al 2006). A phase I clinical trial using lapatinib and weekly trastuzumab has been carried out in 48 patients breast cancer patients with metastatic disease, and one complete response and 5 partial responses have been observed in 27 patients (Ito et al 2007).

In the future, lapatinib will be studied in larger phase III clinical trials. The adjuvant use of lapatinib for early stage ErbB2 over-expressing breast cancer is being conducted in the TEACH trial, where patients receive a one year treatment with lapatinib or placebo following treatment with an anthracycline or taxene containing regimen. Three thousand patients are to be recruited to this trial. The ALTO (adjuvant lapatinib and/or trastuzumab treatment optimization) phase III trial is under development and will involve 8000 patients. ErbB2-expressing breast cancer patients will be treated following chemotherapy with lapatinib or trastuzumab for one year or a sequence of both lapatinib and trastuzumab. These large scale trials will determine the clinical efficacy of lapatinb alone or in combination with trastuzumab (Moy and Goss 2006; Ito et al 2007).

Limitations to ErbB receptor inhibitor therapy (Figure 3)

Trastuzumab is effective in treating breast cancer patients with amplification of ErbB2. Unfortunately, some patients

will not respond to trastuzumab therapy and even in trastuzumab responsive patients, its effectiveness is limited. One of these limitations is drug resistance, and 15% of early stage breast cancer patients with ErbB2 positive tumors will not benefit from adjuvant trastuzumab and develop metastatic disease (Piccart-Gebhart et al 2005; Romond et al 2005). Therefore, some patients have de novo resistance to trastuzumab. Furthermore, patients that initially respond to trastuzumab may develop resistance through acquired or treatment-induced mechanisms. This is a major limitation in the clinical use of trastuzumab. Trastuzumab resistance occurs through multiple mechanisms. It has been shown that trastuzumab does not prevent dimerization of EGF receptors allowing dimers, such as ErbB3/ErbB2, to form. In trastuzumab-resistant cells, it has been demonstrated that ErbB3/ErbB2 dimers may form and permit constitutive signaling to occur (Wehrman et al 2006). Similarly, insulin-like growth factor one receptor (IGF1R) is tyrosine kinase receptor that is over-expressed in breast cancers and binds to ErbB2 in trastuzumab-resistant cells (Nahta and Esteva 2007). This association forms a heterodimer complex that leads to increased activation of the PI3K/AKT and Ras/Erk signaling pathways and subsequent resistance to cell death (Wehrman et al 2006; Ito et al 2007). The most common mechanism in trastuzumab resistance is cleavage of the ErbB2 extracellular domain leaving the intracellular domain intact and capable of signaling. This removes the binding site for trastuzumab without affecting ErbB2 signaling (Xia et al 2004). These mechanisms of resistance reveal the need to develop new targeted therapies that will block or reverse trastuzumab resistance in breast cancer patients.

To combat trastuzumab resistance, new ErbB2 targeted monoclonal antibodies have been developed. Pertuzumab, formerly known as rhuMab-2C4, binds to the extracellular domain II of ErbB2 and blocks the interactions between ErbB2 and other ErbB receptor family members (Nahta et al 2004). In addition, pertuzumab blocks heterdimerization of ErbB2 to IGF1R (Nahta et al 2005). This ability to block ErbB2 hetero-dimerization holds the promise that pertuzumab will overcome trastuzumab resistance in breast cancer patients. In a phase I clinical trial, pertuzumab was found to be well tolerated and clinically active in 21 breast cancer patients with advanced disease. Currently pertuzumab is being combined with trastuzumab in phase II clinical testing for locally advanced breast cancer with ErbB2 over-expression (Johnston et al 2006). In xenograft tumor

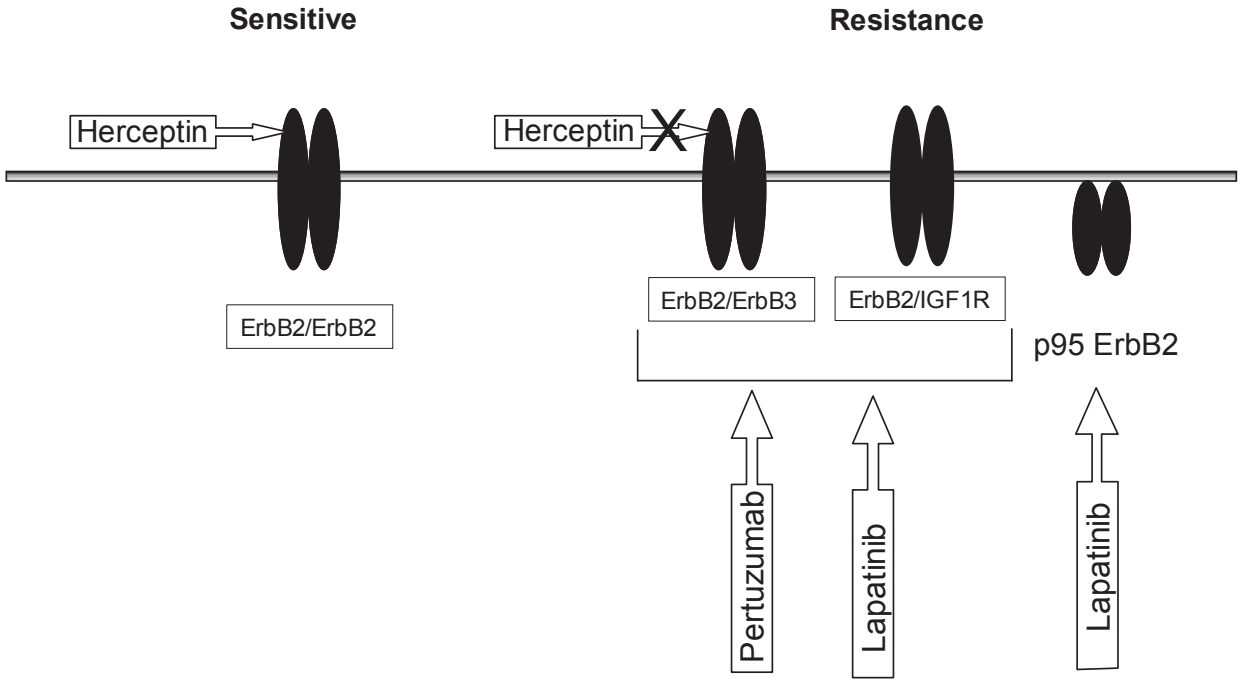

Figure 3 Resistance to trastuzumab in breast cancer. Trastuzumab inhibits the activation of ErbB2 in ErbB2 over-expressing breast cancer cells (sensitive) but many breast cancer patients have or develop resistance to this antibody. This resistance occurs due to the ability of ErbB2 to hetero-dimerize with ErbB3 or insulin growth factor I receptor (IGF1R). In addition ErbB2 is cleaved or alternatively translated into p95 ErbB2 protein lacking the trastuzumab binding sites. This renders breast cancer cells resistant to trastuzumab treatment. Pertuzumab is a monoclonal antibody that prevents ErbB2 dimerization with other receptors and could overcome trastuzumab resistance. Lapatinib inhibits the tyrosine kinase activity in ErbB2 therefore blocks ErbB2 activation when dimerized and in the p95 form.

models of breast cancer, a combination of pertuzumab and trastuzumab increased tumor regression (Arpino et al 2007). In addition to pertuzumab, preclinical studies have also shown that inhibitors of the IGF1R restored sensitivity to trastuzumab in trastuzumab-resistant cells by disrupting the ErbB2/IGF1R interaction (Jerome et al 2006). In addition, IGF1R kinase inhibitors induce apoptosis in trastuzumab resistant cell lines (Nahta and Esteva, 2007). However, these potential treatments for trastuzumab-resistant patients have one major drawback. They will be ineffective against breast cancer cells expressing the p95 cleaved form of ErbB2. Tyrosine kinase inhibitors such as lapatinib are, however, effective at inhibiting that activation of truncated forms of ErbB receptor such as p95 ErbB2. In xenograft breast tumors expressing p95 ErbB2, lapatinib treatment was effective at reducing tumor growth (Xia et al 2004). In cell lines selected for long term outgrowth in trastuzumab containing media, lapatinib still has activity against ErbB receptors (Konecny et al 2006). These results suggest that combining trastuzumab with other ErbB2 inhibitors could reduce trastuzumab resistance.

The use of trastuzumab is also limited by toxicity. In some patients significant cardiotoxicity is observed although this will resolve in 50% of cases after discontinuation of therapy. In the adjuvant setting, grade 3 or 4 congestive heart failure occurs in 4% of patients but most of the symptoms will resolve after therapy is completed. Risk factors for cardiotoxicity are age (>60 years old), concurrent use of anthracyclines (known cardiotoxin) and prior irradiation. The reason for trastuzumab cardiotoxicity is unclear but mice lacking ErbB2 expression have cardiac trabeculae dysfunction (Nahta et al 2007). The most common side effect, however, is skin rash. The most common skin eruptions seen are acneform rash. This rash is different form typical acne by the absence of blackheads or whiteheads and the presence of pruritus (Johnston et al 2006). This side effect may actually be a good sign, since skin epidermal cells have ErbB receptors and the rash indicates that the treatment is effecting ErbB-expressing cells (Herbst 2004). Finally in some patients, hypersensitivity reactions occur with trastuzumab, but these usually occur initially and may not reoccur with subsequent injections (Johnston et al 2006).

Similar to trastuzumab, lapatinib might have limitations. Some breast cancer patients will fail to respond to lapatinib (Geyer et al 2006). It was found in ErbB2 over-expressing early-stage breast cancer patients treated with lapatinib that their biopsied tumor tissue had increased estrogen receptor signaling, as determined by up-regulation of FOXO3a, progesterone receptor (PR) and Bcl-2 (Xia, Bacus et al 2006). This suggests that lapatinib-resistance could be mediated by activation of estrogen receptor signaling. This could also cause these tumors to become endocrine sensitive. Indeed, combining lapatinib and tamoxifen increases apoptosis in estrogen receptor positive breast cancer cells (Chu et al 2005). Lapatinib might also have other clinical limitations. These include the ability of ErbB receptors to mutate their kinase domain rendering the lapatinib ineffective. This was the case for imatinib in chronic myeloid leukemia (CML) patients where mutations in the *bcr-abl* gene casued resistance to imatinib (Mughal and Goldman 2007). Another concern is the dimerization of ErbB2 with IGF1R or ErbB4, where there is tyrosine kinase activity that is not inhibited by lapatinib and/or changes in the PI3K/ATK or Ras/Erk pathways which are independent of ErbB2 activation. It remains to be determined what the mechanism(s) of lapatinib resistance is in breast cancer patients.

Concluding remarks

ErbB receptors are critical regulators of signaling events that can lead to breast cancer progression and are targets for cancer therapy. Trastuzumab has shown success in treating breast cancer but has limitations including development of drug resistance and toxicities. Lapatinib overcomes some of these limitations. Lapatinib targets the intracellular kinase domain of these receptors making loss of extracellular portion of the receptor clinically irrelevant. In addition, lapatinib does not cause the cardiotoxicity seen with trastuzumab (Ito et al 2007). Combining trastuzumab and lapatinib is currently under clinical investigation in an attempt to increase efficacy and minimize toxicity.

Acknowledgments

S.G. is held a new investigator award from the Canadian Institutes for Health Research. M.L. is a Canadian Research Chair in New Cancer Therapeutics.

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