1. Introduction

Approximately 20-30% of breast cancers show increased expression of the HER2 receptor tyrosine kinase. Elevated levels of HER2 are associated with aggressive disease, high metastatic potential, and reduced survival versus other breast cancer subtypes (Slamon, 1987). Trastuzumab (Herceptin) is a monoclonal antibody targeted against an extracellular region of HER2 (Carter, 1992). Clinical trials have shown that 15-30% of patients with HER2-overexpressing metastatic breast cancer respond to single-agent trastuzumab for a median duration of approximately 10 months (Baselga, 1996; Cobleigh, 1999). Response rates improve when trastuzumab is combined with chemotherapy in patients with HER2-overexpressing metastatic breast cancer (Esteva, 2002; Slamon, 2001). A subset of trastuzumab-resistant breast cancers respond to the dual EGFR/HER2 kinase inhibitor lapatinib, although the majority (70% or more) show primary resistance (Geyer, 2006). Similar to trastuzumab treatment, clinical trials with lapatinib indicated that the median duration of response to lapatinib in a heavily pre-treated, trastuzumab-refractory population was less than one year (Geyer, 2006). Hence, resistance to clinically available HER2-targeted agents is a major concern in the treatment of patients with HER2-overexpressing metastatic breast cancer.

2. HER2 and breast cancer

The human epidermal growth factor receptor 2 (HER2) is overexpressed in approximately 25% of invasive breast carcinomas. HER2 is a member of the epidermal growth factor receptor (EGFR) family, which also contains two other receptors, HER3 and HER4 (Fig. 1). Each of these cell surface receptors has an extracellular ligand-binding domain and a transmembrane-spanning domain (Nielsen, 2008). All HER family receptors except HER2 bind specific ligands that induce conformational changes and receptor homo- or heterodimerization. Several HER family ligands have been identified including transforming growth factor alpha (TGFα), epidermal growth factor (EGF), and the heregulins (Nielsen, 2008). In addition, all except HER3 contain an intracellular tyrosine kinase domain. Receptor dimerization activates the kinase function of receptors, leading to receptor auto- or trans-phosphorylation. The phosphorylated tyrosine residues serve as docking sites for SH2 and PTB-domain containing proteins, which links the receptors to multiple cell survival and proliferation pathways including the phosphatidylinositol-3 kinase (PI3K) and mitogen-
activated protein kinase (MAPK) cascades (Spector, 2009; Graus-Porta, 1997). HER2 is the preferred dimerization partner for the other HER family members, as HER2 heterodimers have increased ligand binding affinity and increased catalytic activity relative to other heterodimer complexes (Spector, 2009; Graus-Porta, 1997). In particular, the HER2-HER3 heterodimer has the strongest kinase activity and transforming ability, as HER3 possesses multiple PI3K docking sites in its cytoplasmic tail.

Fig. 1. HER/erbB family of growth factor receptors. The four members of the EGFR family are illustrated. The inactive ligand-binding domains of HER2 and the inactive kinase domain of HER3 are denoted with an X. Trastuzumab binds to domain IV of the extracellular region of HER2.

2.1 Targeting HER2 in breast cancer
Patients who are diagnosed with HER2-overexpressing breast cancer have a poor prognosis, and shorter progression-free and overall survival compared to patients with other subtypes of breast cancer (Eccles, 2001). HER2-overexpressing tumors have been found to be larger in size, and higher in nuclear grade, S phase fraction, and aneuploidy (Nielsen, 2008). Traditional cancer treatments have targeted DNA replication or cell division, leading to nonspecific cytotoxicity (Oakman, 2010). The identification of abnormal signaling from HER2 led to the development of trastuzumab (Herceptin) (Genentech, San Francisco, CA, USA), which is the first drug to target the genetic lesion or oncogenic addiction found in patients with HER2-overexpressing breast cancer. Clinically, trastuzumab was found to significantly enhance the effectiveness of conventional chemotherapies. However, the median duration of response was less than one year, indicating rapid development of
resistance. The precise mechanism of action of trastuzumab is unclear, but it is thought to involve HER2 downregulation (Cuello, 2001; Gajria, 2011), selective inhibition of HER2-HER3 heterodimerization (Junttila, 2009; Gajria, 2011), prevention of HER2 extracellular domain proteolytic cleavage (Molina, 2001; Gajria, 2011), and activation of an immune response including antibody-dependent cellular cytotoxicity (Sliwkowski, 1999). As a single agent, trastuzumab achieved an overall response rate for a median duration of about nine months (Baselga, 1996; Cobleigh, 1999; Nielsen, 2008; Slamon, 2001). The low response rate indicates that many patients with HER2-overexpressing breast cancer have primary resistance to trastuzumab, while the short duration of response indicates rapid development of acquired resistance. Multiple mechanisms contributing to trastuzumab resistance have been proposed, resulting in multiple approaches to potentially treat resistant cancers (Table 1).

<table>
<thead>
<tr>
<th>Target</th>
<th>Role in trastuzumab resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K</td>
<td>Increased PI3K signaling due to PIK3CA mutations or PTEN loss was reported in trastuzumab-resistant cancers</td>
</tr>
<tr>
<td>mTOR</td>
<td>As a downstream molecule of PI3K, mTOR has become a target of inhibition in resistant cancers; multiple mTOR inhibitors are in advanced phases of clinical development</td>
</tr>
<tr>
<td>IGF-IR</td>
<td>Increased expression of IGF-IR has been shown to reduce response to trastuzumab; increased IGF-IR overexpression was associated with lower response to neoadjuvant trastuzumab; IGF-IR/HER2 interaction and crosstalk were associated with acquired resistance</td>
</tr>
<tr>
<td>Src</td>
<td>Trastuzumab-mediated inhibition of Src activity appears to be important to its anti-cancer activity; resistance to trastuzumab was associated with PTEN loss and increased Src activity; targeting Src with dasatinib or genetic knockdown blocked growth of resistant cancers</td>
</tr>
<tr>
<td>Cdk2</td>
<td>Reduced p27kip1 levels or amplification of cyclin E gene have been reported to result in increased cdk2 activity in trastuzumab-resistant cancers</td>
</tr>
</tbody>
</table>

Table 1. Potential pharmacologic targets in trastuzumab-resistant HER2-positive breast cancers.

3. Targeting PI3K/mTOR signaling in HER2-overexpressing breast cancer

HER2 signaling is initiated upon receptor dimerization, which induces phosphorylation of tyrosine residues within the receptor cytoplasmic domain. The phosphorylated residues serve as docking sites for adaptor proteins and link the receptor to downstream survival pathways including the PI3K/Akt/mTOR axis (Spector, 2009). The PI3K pathway is frequently hyper-activated in many cancers. An association between oncogenic PI3K mutations and trastuzumab resistance was found in a study examining HER2-overexpressing tumors from patients with trastuzumab-refractory disease (Berns, 2007). About 25% of tumors analyzed had PIK3CA mutations, and reduced phosphatase and tensin homolog (PTEN) expression was present in 22% of the tumors. Immunohistochemistry studies performed in a retrospective analysis of HER2-amplified breast tumors treated with trastuzumab plus taxanes showed a positive correlation between PTEN down-regulation and tumor response (Nagata, 2004). To evaluate the role of PI3K
post-trastuzumab exposure, tumors that had progressed on trastuzumab were analyzed for changes in PI3K signaling. The findings demonstrated that PI3K mutations and PTEN loss were identified in patients who had initially responded to trastuzumab; reduced PTEN expression was identified in tumors that had developed trastuzumab resistance, but had not been identified before trastuzumab treatment. This finding indicates that PI3K mutations can occur as a result of trastuzumab treatment in some tumors (Kalinsky, 2009; Sakr, 2010; Gajria, 2011). Thus, there is ample rationale for co-targeting PI3K and HER2 in breast cancer. Activated Akt regulates several downstream signaling molecules including mTOR, a highly conserved 289-kDa serine/threonine kinase that plays roles in cell proliferation, survival, and motility (Lang, 2010). mTOR activation is initiated when phosphorylated PI3K/Akt inhibits the TSC1/TSC2 complexes, thereby preventing Rheb from inhibiting mTOR. mTORC1 (mTOR, Raptor, mLST8/GBL and PRAS40) and mTORC2 (mTOR, RICTOR, mLST8/GBL, SIN1, and PROTOR/PRR5) are the two distinct complexes through which mTOR exerts cellular effects. The complexes have different functional roles, with mTORC1 having been implicated in cell cycle progression, motility, and protein biosynthesis, while mTORC2 regulates cytoskeleton organization, and regulates cell growth and survival (Wullschleger, 2005; Van der Heijen, 2011).

Preclinical in vivo studies in which mice were treated with single agent trastuzumab, the mTOR inhibitor rapamycin, or a combination of trastuzumab plus rapamycin showed that the combination was more effective at inducing tumor regression than either of the single agent treatments (Miller, 2009). In cell culture experiments using the rapamycin analogue RAD001, a greater amount of growth inhibition was observed with combination mTOR inhibition plus HER2-targeting than with either drug alone. Trastuzumab partially decreased PI3K activity, but not mTOR activity (Miller, 2009). Increased PI3K signaling is a validated mechanism of trastuzumab resistance, but its association with lapatinib resistance is yet to be determined due to conflicting data (Eichhorn, 2008; O’Brien, 2010). Patients with HER2-overexpressing breast cancer who have developed resistance to trastuzumab may be given the dual EGFR/HER2 tyrosine kinase inhibitor lapatinib. Response to single agent lapatinib is less than 25%, indicating cross-resistance between trastuzumab and lapatinib (Blackwell, 2010; Eichhorn, 2008). As with trastuzumab treatment, the small subset of patients who initially responded to lapatinib eventually developed resistance, at which point there is no standard therapeutic approach available. Phase I trials have indicated that in patients with trastuzumab-resistant, heavily pretreated breast cancer, combined everolimus plus trastuzumab could be a promising treatment (Jerusalem, 2011). It is thought that the inability of trastuzumab to completely inhibit PI3K/Akt/mTOR signaling may permit escape from growth inhibition; mTOR inhibitors would thus synergize with trastuzumab to prevent the continued growth of HER2-dependent cancer cells.

In contrast to PI3K, very little has been published regarding the role of MAPK signaling in trastuzumab resistance. Our data suggests that phosphorylation of Erk1/2, which is a marker of MAPK activity, is not increased in resistant cells (Fig. 2A). Inhibition of MEK (upstream of Erk1/2) using a small molecule MEK kinase inhibitor called PD0325901 reduces p-Erk1/2 levels in parental HER2-overexpressing breast cancer cells and in acquired trastuzumab-resistant and primary trastuzumab-resistant cells (Fig. 2B). However, trastuzumab-naïve and trastuzumab-resistant cells are relatively resistant to PD0325901, in that doses up to 10 uM do not block proliferation of HER2-overexpressing trastuzumab-naïve or resistant cells (Fig. 2C). Thus, our data indicate that MAPK signaling may not be a major mechanism of trastuzumab resistance.
Fig. 2. Role of MAPK signaling in trastuzumab-resistant cells. (A) SKBR3 parental, trastuzumab-resistant pool 2, and BT474 parental, and trastuzumab-resistant clone 2 and clone 3 cells were Western blotted for phosphorylated and total Erk1/2. (B) BT-parental, BT-c2 (resistant clone 2), and MDA-MB-361 primary trastuzumab-resistant cells were treated with MEK inhibitor PD0325901 at 10, 100, or 1000nM for 6 hours or with DMSO control (C) corresponding to the volume found in the highest dose of PD0325901. Total protein lysates were Western blotted for phosphorylated and total Erk1/2. (C) BT-parental, resistant clone 2 and 3, MDA361, and MDA453 cells were treated with MEK inhibitor PD0325901 at 1, 10, 100, 1000, or 10,000nM for 48 hours with six replicates per treatment group. Control cells were treated with DMSO corresponding to the volume found in the highest dose of PD0325901. Proliferation was assessed by MTS assay, and is shown as a percentage of control group per line.

4. Targeting IGF-IR signaling in HER2-overexpressing breast cancer

The insulin-like growth factor receptor I (IGF-IR) is a heterotrimERIC transmembrane tyrosine kinase receptor that regulates cell metabolism and growth (Chaves, 2010), and has
been associated with increased risk and maintenance of multiple cancers including HER2-overexpressing breast cancer (Esparis-Ogando, 2008; Hankinson, 1998; Surmacz, 2000). Circulating ligands of the insulin-like growth factor (IGF) system include IGF-I and IGF-II, with IGF-I having the highest affinity for IGF-IR. Upon binding to IGF-IR, a receptor conformational change is induced that leads to tyrosine phosphorylation and activation of several downstream survival signaling pathways such as the Ras/Raf/mitogen activated protein kinase pathway (MAPK), and the PI3K/Akt/mTOR pathway. Activation of these pathways results in cell cycle progression and resistance to apoptosis (Chaves, 2011; Adams, 2000). The IGF binding proteins (IGFBPs) modulate IGF-IR activity by binding to the IGF ligands thereby sequestering them and preventing ligand-induced receptor activation (Adams, 2000). Higher levels of circulating IGF-I have been linked to trastuzumab resistance in HER2-overexpressing breast cancer, with the addition of IGFBP3 decreasing IGF-IR activity, and subsequently resulting in an increased response to trastuzumab (Lu, 2001; Jerome, 2006).

We found by gene microarray analysis that IGFBP3 and IGFBP5 were down-regulated in resistant versus sensitive cells (Table 2). However, ELISA of secreted IGFBP3 (Fig. 3A) or real-time PCR analysis of endogenous IGFBP3 or IGFBP5 transcript level (Fig. 3B) failed to show any differences in IGFBP3 or IGFBP5 level in resistant versus parental cells. Thus, our data do not support down-regulation of IGFBP3 or IGFBP5 as a mechanism of increased IGF-IR signaling in trastuzumab resistance.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Change</th>
<th>ILMN_GENE</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP5</td>
<td>-20. 55848937</td>
<td>IGFBP5</td>
<td>Homo sapiens insulin-like growth factor binding protein 5 (IGFBP5), mRNA.</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>-20. 0185274</td>
<td>IGFBP5</td>
<td>Homo sapiens insulin-like growth factor binding protein 5 (IGFBP5), mRNA.</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>-7. 77282369</td>
<td>IGFBP3</td>
<td>Homo sapiens insulin-like growth factor binding protein 3 (IGFBP3), transcript variant 2, mRNA.</td>
</tr>
<tr>
<td>PKIA</td>
<td>-6. 484521044</td>
<td>PKIA</td>
<td>Homo sapiens protein kinase (cAMP-dependent, catalytic) inhibitor alpha (PKIA), transcript variant 7, mRNA.</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>-6. 193624741</td>
<td>IGFBP3</td>
<td>Homo sapiens insulin-like growth factor binding protein 3 (IGFBP3), transcript variant 1, mRNA.</td>
</tr>
<tr>
<td>PKIA</td>
<td>-5. 371909749</td>
<td>PKIA</td>
<td>Homo sapiens protein kinase (cAMP-dependent, catalytic) inhibitor alpha (PKIA), transcript variant 6, mRNA.</td>
</tr>
<tr>
<td>BASP1</td>
<td>-4. 444496135</td>
<td>BASP1</td>
<td>Homo sapiens brain abundant, membrane attached signal protein 1 (BASP1), mRNA.</td>
</tr>
<tr>
<td>HERC6</td>
<td>-4. 048474978</td>
<td>HERC6</td>
<td>Homo sapiens hect domain and RLD 6 (HERC6), mRNA.</td>
</tr>
<tr>
<td>FRAS1</td>
<td>-3. 988854857</td>
<td>FRAS1</td>
<td>Homo sapiens Fraser syndrome 1 (FRAS1), mRNA.</td>
</tr>
<tr>
<td>THBS1</td>
<td>-3. 966312615</td>
<td>THBS1</td>
<td>Homo sapiens thrombospondin 1 (THBS1), mRNA.</td>
</tr>
</tbody>
</table>

Table 2. Genes that are down-regulated in SKBR3- and BT474-derived acquired trastuzumab-resistant cells versus parental SKBR3 and BT474 cells by 4-fold or more.
Fig. 3. IGFBP3 and IGFBP5 in resistant and sensitive cells. (A) Secreted IGFBP3 was assessed by ELISA in SKBR3 parental, resistant pool 2, BT474 parental, resistant clone 2 and clone 3 cells. IGFBP3 is shown in pg/mL and was measured in triplicate with reproducible results per line. (B) Real-time PCR analysis of IGFBP3 and IGFBP5 was examined in triplicate per line, with error bars representing standard deviation between replicates. Housekeeping gene RPLPO was measured as an internal control; IGFBP3 and IGFBP5 values are normalized to RPLPO.

A subset of HER2-/ IGF-IR-overexpressing cells were found to be less sensitive to the growth inhibitory effects of trastuzumab when compared to HER2-overexpressing cells that do not overexpress IGF-IR (Lu, 2001). Flow cytometry revealed that after trastuzumab
treatment, HER2 overexpressing cells were less likely to progress through the cell cycle and stopped at the G1 phase, while a greater number of HER2/IGF-IR overexpressing cells passed the restriction point and completed the cell cycle. These results demonstrate that IGF-IR interferes with the growth inhibitory actions of trastuzumab, supporting therapeutic strategies that co-target HER2 and IGF-IR. Further, we discovered that signaling interactions exist between IGF-IR and HER2 in trastuzumab-resistant cancers (Nahta, 2005; Jin, 2008). Immunoprecipitation and immunoblotting experiments revealed that IGF-I stimulation results in an increase in IGF-IR phosphorylation more rapidly in trastuzumab-resistant cells than in trastuzumab-sensitive cells. Furthermore, IGF-IR heterodimerization with HER2 results in HER2 activation in trastuzumab-resistant cells, but not in trastuzumab-sensitive cells, indicating crosstalk between the two receptors. Kinase inhibition or antibody blockade of IGF-IR restores trastuzumab sensitivity. Treatment of trastuzumab-resistant breast cancer cells with the highly specific IGF-IR antibody alpha IR3 disrupted the IGF-IR/HER2 heterodimer and increased trastuzumab sensitivity. These results suggest that IGF-IR-targeted treatments may be useful in combination with trastuzumab.

The association of increased IGF-IR activity with the development of trastuzumab resistance in HER2-overexpressing breast cancer makes IGF-IR an important target. Researchers have been working toward the goal of developing agents that target IGF-IR for the past several years with each generation of agents aimed at producing a greater benefit for the patient while decreasing adverse effects. IGF-IR and the insulin receptor (IR) are 60% homologous, with one of the adverse effects of IGF-IR antibody treatment being downregulation of the IR, leading to hyperglycemia (Sachdev, 2006). In an effort to remedy this problem, pharmacological agents like the small molecule tyrosine kinase inhibitor NVP-AEW541 (Novartis Pharma, Basel Switzerland) are specific for IGF-IR and less likely to interfere with glucose metabolism. Combination treatment with NVP-AEW541 and trastuzumab showed synergistic growth inhibitory effects, indicating that inhibiting IGF-IR plus HER2 could benefit patients whose tumors overexpress both receptors (Esparis-Ogando, 2008).

IGF-IR overexpression and crosstalk with HER2 suggests that IGF-IR plays a crucial role in conferring trastuzumab resistance. The molecular signaling pathways by which IGF-IR confers resistance to trastuzumab is not clear, although downstream focal adhesion kinase (FAK) and PI3K/Akt pathway signaling likely play a role (Yang, 2010). This data linking IGF-IR to the development of trastuzumab resistance, along with the increased sensitivity to trastuzumab upon IGF-IR inhibition provides a rational for the development of combinatorial HER2 and IGF-IR targeting.

5. Targeting Src in HER2-overexpressing breast cancer

Trastuzumab treatment of HER2-overexpressing breast cancer cells results in inhibition of Src non-receptor tyrosine kinase (Nagata, 2004). Src inhibition appears to be important to trastuzumab-mediated anti-cancer activity, as increased Src signaling is associated with trastuzumab resistance (Mitra, 2009; Liang, 2010; Zhang, 2011). One mechanism leading to increased Src activity appears to be a variant of HER2 called HER2 delta 16 (Mitra, 2009), which shows increased oncogenic activity. Local disease progression involved HER2Delta16 in 89% of breast cancer patients with HER2-positive tumors (Mitra, 2009). Transfection of MCF7 or NIH3T3 cells with HER2 delta 16 promoted receptor dimerization, invasion, and trastuzumab resistance (Mitra, 2009). The oncogenic properties of HER2Delta16 were mediated through direct interaction of HER2Delta16 with Src kinase. Activated Src kinase
was found in 44% of HER2Delta16-positive breast carcinomas (Mitra, 2009). Dual targeting of HER2Delta16 plus Src with dasatinib resulted in Src inactivation, destabilization of HER2Delta16, and decreased tumorigenicity (Mitra, 2009). In addition, Src activation via Jak2 has been shown to reduce trastuzumab activity (Liang, 2010). Recombinant human erythropoietin activated Jak2-Src signaling and inactivated PTEN in HER2-positive cells (Liang, 2010). Combined treatment with recombinant human erythropoietin plus trastuzumab reduced response to trastuzumab in cell culture and in vivo models. Further, shorter progression-free and overall survival was found in patients with HER2-positive breast cancer treated concurrently with erythropoietin and trastuzumab (Liang, 2010). Src was also shown to be activated in primary and acquired trastuzumab resistance as a consequence of PTEN loss (Zhang, 2011). Src-targeted therapy blocked growth of trastuzumab-resistant tumors in vivo (Zhang, 2011). Thus, Src activation may occur via multiple mechanisms, ultimately abrogating sensitivity to trastuzumab. Combining Src-targeted therapy with trastuzumab may offer benefit to patients with HER2-overexpressing breast cancer.

6. Role of p27 and cdk2 in HER2-overexpressing breast cancer

Trastuzumab induces G1 arrest by several mechanisms including increased expression of cyclin-dependent kinase inhibitor p27kip1, which inhibits cyclin E/cdk2 and cyclin A/cdk2 complexes and blocks cell cycle progression through S phase (Lane, 2001; Le, 2003). Trastuzumab induces p27kip1 expression by suppressing expression of proteins that sequester p27kip1, which also results in increased interaction between p27kip1 and cdk2 leading to cdk2 inactivation (Lane, 2001). We previously reported (Nahta, 2004b) that cells with acquired trastuzumab resistance showed increased proliferation, reduced p27kip1 expression, reduced p27kip1-cdk2 interaction, and increased cdk2 activity relative to parental, trastuzumab-sensitive cells. Transfection of wild-type p27kip1 increased trastuzumab sensitivity in cells with acquired resistance (Nahta, 2004b). Yakes et al. (Yakes, 2002) showed that knockdown of p27kip1 reduced trastuzumab sensitivity in HER2-overexpressing breast cancer cell lines, further supporting a requirement of p27kip1 expression for optimal response to trastuzumab. Post-translational modification of p27kip1 occurs primarily by phosphorylation, with subsequent protein ubiquitination and degradation. Preliminary data supporting ubiquitin-proteasome degradation of p27kip1 as a mechanism of p27kip1 down-regulation in trastuzumab resistance includes our finding that proteasome inhibitor MG132 induced p27 expression and reduced viability of resistant cells (Nahta, 2004b). Further, Cardoso et al. (Cardoso, 2006) showed that proteasome inhibitor bortezomib induced p27kip1 and increased the efficacy of trastuzumab in HER2-overexpressing breast cancer cells. PI3K inhibition has been shown to induce p27kip1 expression, and is believed to contribute to p27kip1 down-regulation and acquired trastuzumab resistance. In addition to observing reduced p27kip1 levels in models of acquired resistance, our data indicates that p27kip1 expression is down-regulated post-transcriptionally in cells with primary trastuzumab resistance (Fig. 4). Cyclin E expression has been shown to be regulated by HER2 expression status, in that HER2 knockdown resulted in reduced cyclin E level and reduced cyclin E-associated kinase activity (Mittendorf, 2010). In addition, HER2-overexpressing breast cancers that also show increased cyclin E expression have lower 5 year disease-free survival versus those that have lower cyclin E levels (Mittendorf, 2010). Recently, cyclin E overexpression
in HER2-overexpressing breast cancer cells that have acquired trastuzumab resistance was shown to be due to amplification of the cyclin E gene (Scaltriti, 2011). Amongst 34 patients with HER2-overexpressing breast cancer, cyclin E amplification was associated with worse response to trastuzumab (Scaltriti, 2011). Knockdown of cyclin E or cdk2 inhibition reduced proliferation and induced apoptosis of trastuzumab-resistant tumors (Scaltriti, 2011). Thus, cdk2 inhibition is a potential pharmacologic strategy for treating trastuzumab-resistant HER2-overexpressing breast cancers that show reduced p27kip1 or increased cyclin E levels.

Fig. 4. p27 down-regulation in models of intrinsic (primary resistance). (A) SKBR3 and BT474 trastuzumab-sensitive cells and trastuzumab-resistant HCC1419, HCC1954, and JIMT-1 cells were examined by Western blotting for p27 and actin internal control. (B) BT474 and acquired resistant clone BT-HRc1 and primary resistant HCC1954 and JIMT-1 cells were examined by real-time PCR for p27 transcript which was normalized to RPLPO housekeeping gene.

7. Combining multiple HER2-targeted agents in HER2-overexpressing breast cancer

Two HER2-targeted agents are currently approved for use in the setting of metastatic HER2-positive breast cancer, trastuzumab and lapatinib. These agents target HER2 via distinct mechanisms (Fig. 5). Trastuzumab is a monoclonal antibody that specifically recognizes and binds to an extracellular part of HER2. Since antibodies are large, bulky molecules, trastuzumab is unable to cross the blood-brain barrier and thus cannot combat brain metastases. In contrast, lapatinib is a small molecule kinase inhibitor targeted against the EGFR and HER2 active sites. Since it is a small molecule, it is believed that lapatinib has the potential to enter the brain and target metastatic cells that overexpress HER2. A phase II trial of lapatinib in patients with trastuzumab-refractory disease and CNS metastases showed some volumetric changes in brain lesions and improved neurologic symptoms (Lin, 2008; Lin, 2009). Amongst 50 patients who were treated with lapatinib plus capecitabine, 20% showed a CNS objective response and 40% experienced 20% or greater volumetric reduction in their CNS lesions (Lin, 2009), suggesting that lapatinib may have some utility in limiting CNS metastases of primary HER2-overexpressing breast cancers.
7.1 Combining trastuzumab with lapatinib
Combination of trastuzumab plus lapatinib has been shown to induce apoptosis in part via down-regulation of survivin in cell culture and animal models (Xia, 2005). Initial phase I data suggested that the combination is well-tolerated and elicits partial or complete responses in a subset of patients who have progressed on prior trastuzumab therapy (Storniolo, 2008). The combination has been tested clinically in advanced phase trials in patients who have progressed on trastuzumab-based regimens. Progression-free survival and quality of life were improved in patients treated with the combination versus lapatinib alone (Wu, 2011). EGF104900 showed that the combination was superior to lapatinib alone in the trastuzumab-resistant setting, with a clinical benefit rate of 24.7% versus 12.4% (Blackwell, 2010). A potentially important mechanism of action of this drug combination is that lapatinib has been shown to induce accumulation of inactive HER2 dimers via reduced receptor ubiquitination, providing increased pharmacologic target for trastuzumab-mediated antibody-dependent cellular cytotoxicity (Scaltriti, 2009). Combining trastuzumab with lapatinib offers a chemotherapy-free option for treating HER2-positive trastuzumab-resistant disease.

7.2 Combining trastuzumab with pertuzumab
Pertuzumab is an anti-HER2 monoclonal antibody that targets an extracellular epitope distinct from what is targeted by trastuzumab. Pertuzumab binds to HER2 near the center of
domain II, sterically blocking a binding pocket necessary for receptor dimerization and signaling (Franklin, 2004). In contrast, trastuzumab does not significantly inhibit HER2 interaction with other erbB receptors. We were the first to show that combining pertuzumab with trastuzumab results in synergistic inhibition of proliferation of HER2-overexpressing breast cancer cells (Nahta, 2004a). Trastuzumab increased pertuzumab-mediated disruption of HER2 dimerization with EGFR and HER3, and further reduced pertuzumab-mediated inhibition of PI3K signaling (Nahta, 2004a). Phase II data shows that combining trastuzumab with pertuzumab in patients who have progressed on prior trastuzumab regimens achieves clinical benefit rate of 50%, objective response rates of 24%, and median progression-free survival of 5.5 months (Baselga, 2010a). A potential mechanism of synergy is non-overlapping mechanisms by single agents, trastuzumab-mediated inhibition of p95HER2 cleavage and pertuzumab-mediated disruption of dimerization (Scheuer, 2009). Clinical evaluation of pertuzumab and trastuzumab (CLEOPATRA) is an international, randomized, double-blind, placebo-controlled phase III trial. Patients with HER2-positive breast cancer with locally recurrent or metastatic disease will be randomized to receive docetaxel, trastuzumab, and pertuzumab or docetaxel, trastuzumab, and placebo. Progression-free survival will be assessed to determine efficacy of combination pertuzumab plus trastuzumab in the trastuzumab-refractory setting (Baselga, 2010b).

8. Novel HER2-targeted agents in clinical development

8.1 Trastuzumab-DM1
One novel preparation of trastuzumab is a drug conjugate called trastuzumab-DM1, which is trastuzumab conjugated to a microtubule-depolymerizing drug called maytansinoid (Lewis Phillips, 2008). Trastuzumab-DM1 blocks growth of trastuzumab-naïve and trastuzumab-refractory HER2-overexpressing breast tumors in vivo (Lewis Phillips, 2008), and retains the mechanistic activity of unconjugated trastuzumab (Junttila, 2010). Antibody-dependent cellular cytotoxicity was induced by trastuzumab-DM1, and tumor growth of trastuzumab-resistant cells was blocked by trastuzumab-DM1 due to induction of apoptosis and mitotic catastrophe (Barok, 2011). A phase I dose-escalation study in patients who had progressed on trastuzumab showed clinical benefit of 73% in 15 of 24 patients, including objective responses in 5 patients (Krop, 2010). A phase II study of trastuzumab-DM1 in patients with trastuzumab-refractory HER2-positive breast cancer showed objective response of 25.9% and median progression-free survival of 4.6 months (Burris, 2011). Thus, trastuzumab-DM1 HER2 antibody-chemotherapy conjugate is a promising treatment for HER2-positive breast cancer that has progressed on prior HER2-directed therapies.

8.2 Irreversible pan-HER kinase inhibitors
In contrast to lapatinib, which is a reversible EGFR/HER2 kinase inhibitor, irreversible pan-HER inhibitors are being developed for use against HER2-dependent breast cancers (Ocana, 2009). Neratinib, an irreversible EGFR/HER2 inhibitor, achieved a response rate of 26% in trastuzumab-pretreated patients and 55% in trastuzumab-naïve patients (Burstein, 2009). Progression-free survival at 16 weeks was 60% and 77%, respectively, for trastuzumab-pretreated and naïve patients (Burstein, 2009). Finally, the median time to progression was 23 weeks and 40 weeks, respectively, for trastuzumab-pretreated and naïve patients (Burstein, 2009). Canertinib (CI-1033) is an irreversible inhibitor of all HER proteins. Response to canertinib was higher in patients with HER2-positive breast cancer, although toxicity at the most effective dose was limiting and unacceptable (Rixe, 2009).
9. Conclusion

In conclusion, several major mechanisms of trastuzumab resistance have been proposed, including increased signaling from PI3K/mTOR, Src, and IGF-IR, as well as reduced p27kip1 and increased cdk2 activity. These mechanisms have uncovered new therapeutic targets for which multiple pharmacologic agents have been developed. Some of the most promising include mTOR-targeted agents derived from rapamycin and trastuzumab-DM1. Combining multiple HER2-targeted agents appears to be beneficial due to different mechanisms of action. Future studies should more clearly address the role of IGF-IR in acquired versus primary resistance, and test IGF-IR-targeted agents in combination with trastuzumab and/or lapatinib in a trastuzumab-refractory setting. In addition, studies examining the role of estrogen receptor (ER) signaling in trastuzumab-resistant HER2-positive ER-positive disease should be performed. Finally, biological predictors of response or resistance need to be developed to determine which patients are most likely to benefit from trastuzumab therapy, thus allowing for more specific individualization of targeted therapy in patients with HER2-overexpressing breast cancer.

10. References


Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the


www.intechopen.com


Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:
