Cancer Chemother Pharmacol (2010) 65:611–623
DOI 10.1007/s00280-009-1208-1

MINI REVIEW

Progression and treatment of HER2-positive breast cancer

April Davoli · Barbara A. Hocevar · Thomas L. Brown

Received: 19 August 2009 / Accepted: 4 December 2009 / Published online: 20 December 2009

Abstract
Purpose Approximately 20–30% of breast cancer tumors overexpress or amplify human epidermal growth factor receptor 2 (HER2). The role of this receptor in the progression of HER2+ breast cancer and resistance to certain anticancer monotherapies was investigated. The results of several pre-clinical and clinical trials, with the aim of determining the most safe and effective course of treatment for HER2+ breast cancer, were also thoroughly examined.
Methods A thorough search of databases including Pubmed, Springer, and The American Society of Clinical Oncology was performed, and pertinent studies were identified. The most relevant studies were preclinical, phase II, and III clinical trials identifying the function of the HER2 receptor in HER2+ breast cancer progression, as well as studies assessing the efficacy of monotherapy and combination therapy in the treatment of this aggressive form of cancer.
Results The HER2 receptor belongs to a family of receptors that consists of four cell-surface receptors (HER1-4) that share strong homology with the epidermal growth factor receptor (EGFR). All HER receptors interact with specific types of ligands to induce receptor activation, except for HER2, for which no known ligand has yet been identified. HER2 is activated by forming dimers with other HER receptors, and this results in a stronger and more prolonged signal transduction event. When expressed at normal levels, HER2 regulates cell growth, differentiation, and survival. However, under pathological conditions of HER2 overexpression, numerous HER2 heterodimers are formed resulting in aggressive tumor growth. Therefore, the prognosis associated with HER2-positive breast cancer is usually poor. A specific cohort of patients with breast cancer whose tumors test both hormone receptor (estrogen receptor [ER] and progesterone receptor [PR]) and HER2 positive have been found to be resistant to targeted hormone therapy. Studies investigating the etiology of this resistance have found that the cell membrane estrogen receptor communicates with HER2 in promoting the release of ER coactivators that cause the endocrine drug and selective estrogen receptor modulator, tamoxifen, to act as an agonist rather than an antagonist of the hormone estrogen. Thus, research has directed its inquiry toward the development of therapies specifically targeting HER2. The development of trastuzumab, a recombinant monoclonal antibody against HER2, initially proved to be a well-tolerated first line of treatment. However, in the long-term patients, trastuzumab was shown to develop resistance to this monotherapy. Therefore, research on HER2 positive breast cancer has focused on the study of different anti-HER2 combination therapies over the past decade.
Conclusions While the development and approval of the HER2-targeted recombinant monoclonal antibody trastuzumab (Herceptin) has been efficacious in slowing HER2 cancer progression, combining this and other anti-HER2 therapy with either chemotherapy or endocrine therapy has proven more effective in improving overall and progression free survival.

A. Davoli · T. L. Brown (✉)
Laboratory of Cell Death, Differentiation, and Development,
Department of Neuroscience, Cell Biology, and Physiology,
Wright State University Boonshoft School of Medicine,
3640 Colonel Glenn Highway, 011 Medical Sciences Building,
Dayton, OH 45435, USA
e-mail: thomas.L.brown@wright.edu

B. A. Hocevar
Department of Pharmacology and Toxicology,
Indiana University School of Medicine,
Indianapolis, IN 46202, USA

Keywords HER2 · Trastuzumab · Hormone receptor · Crosstalk · Combination therapy

Introduction

In the year 2008, more than 180,000 new cases of breast cancer were diagnosed in the United States alone. Although modern medicine has progressed in finding new treatments for breast cancer, nearly 41,000 of these new cases resulted in death, of which 99% of these deaths occurred in women. In women, breast cancer is the most commonly diagnosed type of cancer, accounting for 26% of all new cancer cases and is the second most deadly accounting for 15% of cancer-related deaths [1]. Over half of these cases are hormone receptor positive, a status amenable to targeted hormone therapy. However, both primary and secondary resistance to hormone therapy in patients with hormone receptor (HR) positive tumors has become an increasing complication [2]. Several explanations have been proposed as to why some HR-positive tumors initially have this resistance or later develop resistance to hormonal treatment. One proposed mechanism in conferring intrinsic resistance to hormone therapy implicates the human epidermal growth factor family of receptors (HER), specifically the HER2 family member, through its overexpression and/or amplification in hormone receptor positive tumors [3, 4].

The HER/EGF receptor family is a family of receptors comprised of four cell-surface receptors, HER1, HER2, HER3, and HER4 [5, 6]. The amino acid sequences of the HER family are highly homologous to the epidermal growth factor receptor (EGFR); therefore, the genes for the HER family of receptors are designated erbB1, erbB2, erbB3, and erbB4 [7, 8]. These receptors function in the regulation of cell growth, differentiation and survival. Receptor activation requires three components: a ligand, a receptor, and a dimerization partner. When a specific ligand binds to a HER receptor, it must combine with another receptor of similar structure and undergo dimerization. This initiates a cascade of phosphorylation and signal transduction events that ultimately affect the transcription of specific genes involved in cell proliferation and survival [8].

HER receptors can assemble into different combinations to form both homodimers and heterodimers. All HER receptors interact with specific ligands to induce receptor activation, except for HER2 (erbB2), for which no natural ligand has yet been identified [5, 6, 8]. However, a variety of different growth factors (such as EGF, TGFα, amphiregulin, heparin-binding EGF-like growth factor, betacellulin, epiregulin, and neuregulins) serving as ligands for HER1 (erbB1), HER3 (erbB3), and HER4 (erbB4) can trigger rapid receptor dimerization with marked preferential recruitment of HER2 into a heterodimeric complex. These HER2 (erbB2) containing dimers produce a stronger and more prolonged signal transduction event, in comparison with other HER2 deficient combinations that signal more weakly [5, 6, 8]. Therefore, when HER2 (erbB2) is overexpressed and/or amplified in certain cell types, its sensitivity to growth factors increases and the cell can proliferate unabated resulting in tumor growth [6].

The HER2/neu gene (also known as erbB2) is a proto-oncogene localized to chromosome 17q21. This gene encodes a 1,255 amino acid, 185-kDa transmembrane glycoprotein (also entitled $p185^{HER}$), with intrinsic tyrosine kinase activity. The intracellular domain of HER2 contains a terminal carboxyl segment with the intrinsic ability to autophosphorylate [5, 6]. The activity of this segment accounts for the transmission of the extracellular signal into an intracellular signal transduction event with physiologic repercussions. HER2 has been found to be overexpressed in 20–30% of breast cancer tumors [6].

Determination of breast tumor HER2 status is of importance in determining an optimal breast cancer treatment. All modes of testing correlate a poor prognosis with HER2 overexpression or amplification [3]. Tumors overexpressing HER2 have been found to be more aggressive and result in a higher mortality rate in comparison with tumors that do not [6, 9]. Interestingly, current data positively correlates HER2 overexpression with the challenge of hormone therapy resistance. In addition, this data has shown that HER2 overexpression results in an increase in tamoxifen resistance, a drug commonly used in adjuvant hormone therapy [10–12]. These data suggest that HER2 overexpression may play a role in conferring intrinsic resistance to anti-hormone therapy, which in some individuals renders it impotent as a single therapy. This has directed research toward the use of different combination therapies in the treatment of HER2+/HR+ breast cancers.

Common methods for evaluation of HER2 status are quantitative immunohistochemistry (QIHC), specifically with the Dako 'Herceptest,' as well as CB11, a HER2/neu monoclonal antibody, and by fluorescence in situ hybridization (FISH). Tumors that test negative (low/normal levels) for HER2, according to the IHC Herceptest, are assigned scores of 0 to 1+ and those testing positive for HER2 overexpression and/or amplification score 2+ to 3+ on the 0 to 3+ scale. FISH evaluates the ratio of HER2 to chromosome 17 copy number in carcinoma cells. Normal cell levels of HER2 (HER2 negative cancer cells) exhibit a ratio of less than 2 and a ratio of greater than 2 is interpreted as gene amplification (HER2 positive cancer cells). Many major studies have strictly used IHC for HER2 patient selection. However, more recent studies have found that FISH is a more precise, accurate, and reproducible test for HER2 status, even among patients with tumors that stain poorly and are thus more difficult to evaluate [3].

Crosstalk between ER and HER2/neu

A mechanism for tamoxifen resistance

The development of endocrine therapy has proven beneficial for many patients diagnosed with HR+ breast cancer. However, the use of endocrine therapy has become increasingly limited due to primary resistance or the development of secondary resistance [2]. Only about one half of estrogen receptor (ER+) tumors are amenable to endocrine therapy upon initial treatment with antiestrogens like tamoxifen [13, 14]. Unfortunately, a significant number who respond to this initial treatment develop secondary resistance. This reaction allows the cancer to progress unabated leading to metastatic tumor growth and death [13, 14]. Understanding the mechanisms by which ER+ tumors establish resistance to antiestrogen treatments is of great importance in further promoting the survival of patients with breast cancer.

The ER has both genomic and nongenomic activities and is largely found within the cell's cytoplasm, but some ER can also reside within the nucleus or the cell membrane. The binding of estradiol (E2) to ER results in receptor phosphorylation, dimerization, and the recruitment of coregulatory proteins to promoter regions of estrogen-responsive genes (genomic action) [12, 14]. Coregulatory proteins can either function as coactivators, which facilitate the binding of transcription factors to target DNA sequences and subsequent connections with transcriptional machinery, or corepressors, such as NCoR (Nuclear hormone receptor Co-Repressor) and SMRT (Silencing Mediator of Retinoic acid and Thyroid hormone receptors), which regulate gene expression by binding to a specific DNA sequence and decreasing transcription, thus blocking gene expression. The induced conformational change of estrogen-bound ER promotes the recruitment of coactivators such as A1B1 (Amplified in Breast Cancer 1) or SRC1 (Steroid Receptor Coactivator 1) that favor the expression of genes that function in cell proliferation and survival. In contrast, the induced conformational change of tamoxifen-bound ER promotes the recruitment of corepressors, such as, NCoR and histone deacetylase 3, which can inhibit the expression of prosurvival genes [12].

Interestingly, it has been found that, depending upon the tissue, tamoxifen can function as either an agonist or antagonist of gene transcription. This difference in tamoxifen function is thought to be dependent upon the differential expression of coactivators and corepressors within the tissue microenvironment [12]. An in vitro study that sought to establish whether coregulatory proteins could modulate antiestrogen activity found that higher levels of coactivators like A1B1 (SRC 3) and SRC1 amplify the agonist activities of tamoxifen [15].

In addition to the ER genomic activities, recent evidence has led researchers to postulate that the cell membrane ER communicates with epidermal growth factor receptor (EGFR) and HER2/neu resulting in activation of these receptors. It is thought that this cell signaling crosstalk enhances the growth promoting effects of estrogen (nongenomic activity; Fig. 1), [16].

The cell membrane–associated ER is a G protein–coupled receptor (GPCR). The activity of G protein subunits, $G\alpha_i$, $G\alpha_q$ and $G\beta\gamma$, has been found to be involved in the activation of EGFR, which ultimately regulates gene expression and cell growth. Estradiol, the ligand for these G protein–coupled ERs, is a rapid activator of a signal transduction cascade that transactivates EGFR. It has been proposed that elements within the E domain of the ER allow for the complex interactions with the G proteins, Src tyrosine kinase, and other signaling molecules [16]. The E domain is one of 6 ER domains involved in ligand binding that leads to transcriptional activation. The Src kinase, as well as the matrix metalloproteases MMP-2 and MMP-9, has been found to play an integral role in EGFR transactivation. Following E2/ER binding, a complex interaction occurs between ER-associated G protein subunits and Src. The G protein subunit complex, $G\alpha_i$, $G\alpha_q$ and $G\beta\gamma$, activates Src. This interaction has been proposed to be mediated by the activity of several other proteins such as phospholipase C (PLC), protein kinase C (PKC), and 1,4,5-inositol/triphosphate (IP3). Once activated, Src is necessary for the subsequent activation of MMP-2 and MMP-9. The involvement of MMPs is not surprising, given that these proteins have previously been implicated in the aggressive behavior of breast cancer cells by playing a role in cell migration, invasion, and metastasis. Following activation, it has been found that MMPs induce the secretion of heparin-bound epidermal growth factor (HB-EGF), which then transactivates the EGFR [16].

Stimulation of EGFR leads to the activation of the MAPK pathway and subsequent phosphorylation of extracellular signal-related kinase 1 (ERK1) and ERK2 (MAPK) [12, 16]. Since the MAPK pathway has been found to be responsible for the phosphorylation and thus activation of both ER and the ER coactivator A1B1, this can thus promote the expression of genes that favor cell proliferation and survival. Therefore, MAPK may be modulating the activity of ER coactivators in the final response to growth factors [12, 17]. Interactions between EGFR and HER2 and their downstream activation of ERK show that both receptors make quantitatively equivalent contributions to the transient amplification of ERK in human mammary epithelial cells [18]. Therefore, both EGFR and HER2 act synergistically to cause aberrant tumor growth.

Recent research supports the hypothesis that patients whose tumors express high levels of HER2/neu and ER

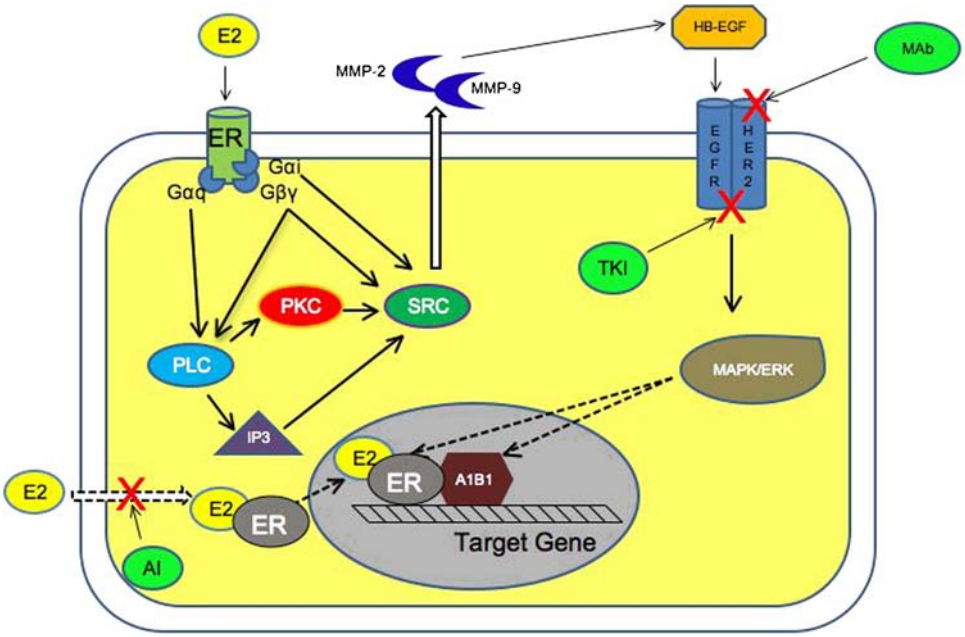

Fig. 1 Nongenomic Signaling of the Cell Membrane ER in HER2 Activation. The cell membrane G protein–coupled ER has been shown to communicate with EGFR and HER2 resulting in activation of these receptors which in turn enhances the growth promoting effects of estrogen. Upon signaling from estradiol (*E2*), ER G proteins, Gαi, Gαq, and G$\beta\gamma$ activate SRC. Activation of SRC by these G proteins is mediated by PLC, PKC and IP3. Once activated SRC then subsequently goes onto activate MMP-2 and MMP-9. Once activated MMPs go onto induce the secretion of HB-EGF, which acts as the ligand for EGFR. Stimulation of EGFR leads to preferential heterodimerization with HER2 and activation of the MAPK/ERK1 pathway. The MAPK pathway is then responsible for the phosphorylation and activation of ER and ER coactivator A1B1. This pathway leads to increased growth and cell proliferation. *ER* estrogen receptor, *EGFR* epidermal growth factor receptor, *HER2* human epidermal growth factor receptor 2, *E2* Estradiol, *SRC* steroid receptor coactivator, *PLC* phospholipase C, *PKC* protein kinase C, *IP3* 1,4,5-inositol/triphosphate, *MMP-2* (& −9) Matrix Metalloprotease-2 (& −9), *HB-EGF* heparin bound-epidermal growth factor, *MAPK* mitogen activated protein kinase, *ERK1* extracellular signal-related kinase 1, *A1B1* amplified in breast cancer 1, *MoAb* monoclonal antibody, *TKI* tyrosine kinase inhibitor, *AI* aromatase inhibitor

coactivator A1B1 will develop tamoxifen resistance. MCF-7 breast cancer cells, expressing high levels of HER2/neu and A1B1, treated with tamoxifen were growth stimulated. When pretreated with gefitinib, an EGFR tyrosine kinase inhibitor, however, receptor crosstalk in the MCF-7/HER2 cells was found to be inhibited, leading to the restoration of tamoxifen's antitumor function. These studies thus suggest that in tumors displaying HER2 amplification and increased A1B1 levels, as in MCF7 cells, tamoxifen behaves as an estrogen agonist resulting in metastatic tumor growth. By interrupting the receptor crosstalk, gefitinib blocks the agonist activity of tamoxifen, allowing it to regain its estrogen antagonist properties [12]. To further support these findings, it was recently reported that patients with breast cancer displaying high Her2/neu and A1B1 levels experience cancer relapse more frequently than those whose tumors are ER+ but do not over express HER2 and/or A1B1 [11]. Together, these studies underscore the association of HER2 amplification and high A1B1 levels with tumor infiltration and poor prognosis. In addition, these studies suggest that combination therapy involving anti-HER2/antiestrogen therapy may provide improved efficacy in the treatment of HER2/ER positive breast cancer.

The efficacy of monotherapies in HER2 positive breast cancer

The use of different biologic therapies to treat metastatic breast cancer, especially HER2+ breast cancer, has grown since the approval of trastuzumab (Herceptin) in 1998 by the US Food and Drug Administration (FDA). In addition to the use of monoclonal antibodies, such as trastuzumab to treat HER2+ breast cancer, clinical trials using tyrosine kinase inhibitors (lapatinab & gefitinib), as well as the development of vaccines against HER2/neu, have been promising in slowing HER2+ tumor progression (Table 1) [12, 19–21].

Trastuzumab was developed by Genetech Inc. and was the first FDA approved monoclonal antibody targeting HER2 overexpression in breast tumors. The 1999 study by Cobleigh and colleagues showed that trastuzumab rarely produced the adverse side effects seen with chemotherapy treatment such as alopecia, mucositis and neutropenia. In addition, out of the 222 women with HER2 overexpressing metastatic breast cancer that had progressed after chemotherapy, an overall response rate of 15% (95% confidence interval, 11–21%) was observed. Upon final evaluation,

Table 1 Method of action of drugs reviewed

Name	Drug	Type	Action
Tamoxifen	ER antagonist	Selective ER modulator	Inhibits the binding of E2 to estrogen receptors and reduces DNA synthesis
Trastuzumab	Anti-HER2	Humanized MoAb	Binds to HER2 blocking its downstream signaling pathway; promotes HER2 endocytotic degradation
Pertuzumab	Anti-HER2	Humanized MoAb	Binds to HER2 and prevents heterodimerization
Trastuzumab (DM1)	Anti-HER2	Immunoconjugate	Direct delivery of chemotherapeutic agent to cancerous tissue
Lapatinib	Anti-HER2/EGFR	Small molecule TKI	Inhibits TK autophosphorylation Blocks intracellular signal transduction of EGFR and HER2
Gefitinib	Anti-HER2/EGFR	TKI	Inhibits TK autophosphorylation Blocks intracellular signal transduction of EGFR and HER2
Neratinib	Anti-HER2/EGFR	Pan-erb TKI	Irreversibly inhibits TK autophosphorylation and transmission of intracellular signal of HER1, HER2, and HER4
Fadrozole	Anti-estrogen	Nonsteriodal AI	Inhibits aromatase by blocking aromatization of androstenedione and testosterone to estrone and estradiol
Letrozole	Anti-estrogen	Nonsteriodal AI	Inhibits aromatase by blocking the aromatization of androstenedione and testosterone into estrone and estradiol
Anastrozole	Anti-estrogen	Nonsteriodal AI	Inhibits aromatase Blocks the aromatization of androstenedione and testosterone into estrone and estradiol
Cyclophosphamide	Chemotherapeutic	Synthetic alkylating agent	Alkylates DNA and produces interstrand DNA crosslinks that inhibit DNA replication
Capecitabine	Chemotherapeutic	5-FU prodrug	Converted to 5-fluorouracil. 5-FU metabolites inhibit DNA and cell division, and inhibit RNA and protein synthesis
Fluorouracil	Chemotherapeutic	Antimetabolite fluoropyrimidine analog	Metabolites inhibit DNA and cell division, and inhibit RNA and protein synthesis
Vinorelbine	Chemotherapeutic	Ditartrate salt of a vinca alkaloid	Inhibits tubulin polymerization into microtubules; antimitotic
Gemcitabine	Chemotherapeutic	Deoxycytidine nucleoside analog	Metabolites inhibit ribonucleotide reductase, thus inhibiting DNA synthesis
Epirubicine	Chemotherapeutic	Anthracycline	Intercalates into DNA to inhibit TopoI and DNA replication and repair
Methotrexate	Chemotherapeutic	Antifolate agent	Binds to and inhibits dihydrofolate reductase leading inhibition of DNA and RNA synthesis
Docetaxel	Chemotherapeutic	Taxane	Binds to and stabilizes tubulin, inhibits microtubule disassembly leading to G2/M phase cell cycle arrest and apoptosis
Cisplatin	Chemotherapeutic	Platinum salt	Form DNA-platinum adducts to crosslink DNA and induce apoptosis
Carboplatin	Chemotherapeutic	Platinum salt	Form DNA-platinum adducts to crosslink DNA and induce apoptosis

ER estrogen receptor, *E2* estradiol, *MAb* monoclonal antibody, *TK* tyrosine kinase, *TKI* tyrosine kinase inhibitor, *AI* aromatase inhibitor, *5-FU* 5 fluorouracil, *TopoII* DNA topoisomerase II, *EGFR* epidermal growth factor receptor, *HER 1,2, or 4* human epidermal growth factor receptor 1,2, or 4

approximately one quarter of responders had no signs of treatment failure with an 82% survival rate [19].

The efficacy of trastuzumab, both with or without prior neoadjuvant or adjuvant chemotherapy, supports its use as a well-tolerated and active first-line of treatment in women with weak positive (2+) and especially in complete positive (3+) HER2 metastatic breast cancer [20–23]. However, the risk of cardiac dysfunction manifested as congestive heart failure, cardiomyopathy, decreased ejection fraction, and ventricular arrhythmia appears to be increased if the patient has been treated with anthracyclines or has a history of cardiac disease prior to trastuzumab treatment [20–22]. Although this incidence of cardiac dysfunction is relatively low, previous exposure to anthracyclines should be treated

as a risk factor and qualified patients should be monitored with caution [20, 24].

Tyrosine kinase inhibitors, such as lapatinib and gefitinib, have been relatively successful in stopping disease progression in patients unresponsive to trastuzumab or chemotherapy alone regimens [12, 25]. Both lapatinib and gefitinib bind to intracellular domains of EGFR and HER2, blocking the activation of downstream MAPK signaling [26]. A phase II, clinical study assessing lapatinab monotherapy in chemotherapy refractory HER2+ and HER2— advanced metastatic breast cancer—showed that 6% of patients benefited clinically from lapatinib treatment. These patients remained progression-free for ≥6 months [25]. A different phase II multicenter study assessing the efficacy of gefitinib in patients unresponsive to chemotherapy pretreatment showed that gefitinib monotherapy was well tolerated, but not efficacious in producing complete or even partial responses [27]. These differences may be explained by prior assessment of patient HER2 status, which was performed in the lapatinib but not the gefitinib trial. Together, these trials demonstrate the need for testing in order to determine HER2 overexpression and/or amplification due to its ability to predict treatment response.

A more recent study evaluating lapatinib as a first-line therapy in HER2+ metastatic breast cancer determined that the drug was quite effective in halting disease progression. Approximately 24% of 138 patients treated with lapatinib for 17.6 weeks displayed an overall response with 31% deriving some clinical benefit. In addition, progression-free survival rates were 63 and 43% at 4 and 6 months, respectively [28]. Therefore, treatment with tyrosine kinase inhibitors such as lapatinib has proven to be clinically active and tolerable, warranting its further investigation as a first-line therapy in adjuvant HER2+ breast cancer.

An emerging form of biologic therapy using peptide vaccinations that target HER2/neu oncoprotein has been shown to be beneficial as an early stage disease treatment in patients with intact immune systems [29]. Currently, preliminary tests are being performed in order to determine whether these patients will develop immunity to both HER2/neu peptides and protein. The goal of these tests is to secure their safety as well as to develop an effective immune response to HER2/neu [30]. Researchers have experienced some success in producing vaccines that have generated T-cell immunity to HER2/neu peptide and protein in the majority of patients treated (≤89%). These studies have also reported retention of HER2/neu specific T cell immunity up to 1 year following treatment cessation [30, 31]. However, few clinical trials have been performed that test the ability of vaccines to slow or eliminate disease progression. Extensive research is needed to test the clinical utility of cancer vaccinations in general as well as those specifically targeting HER2/neu oncoprotein.

Endocrine therapy for patients whose breast tumors express hormone receptors can either act by modulating the activity of the ER or by decreasing circulating hormone levels. Tamoxifen, a selective estrogen receptor modulator, has been the drug of choice for adjuvant endocrine treatment for more than two decades [32]. However, the issue of tamoxifen resistance has complicated the treatment of hormone sensitive breast cancer. As previously noted, the expression of HER2 oncoprotein has been implicated in conferring resistance to hormone therapy, thus necessitating the exploration of alternative forms of endocrine therapy for HR+/HER2+ tumors [2].

Many current studies are also testing the efficacy of estrogen receptor modulators like tamoxifen against aromatase inhibitors (AIs) such as fadrozole, letrozole, or anastrozole that act by inhibiting the production of estrogen. In a double-blinded study testing the response of postmenopausal women to different forms of endocrine therapy, the response of HR+ tumors to letrozole was 60% with 48% having undergone successful breast conserving surgery [33]. However, the response of HR+ tumors to tamoxifen was 41% with only 36% breast conservation. The contrast between the results of these two treatments was most marked in tumors that overexpressed HER1, HER2, and ER. Tumors that were positive for HER1 and HER2 responded successfully to letrozole treatment but not to tamoxifen. This study showed that the signaling crosstalk between HER1 and HER2 through the ER is dependent upon estrogen. Therefore, estrogen deprivation through the use of AIs proved to be a more successful anti-tumor treatment in these patients.

Treatment with AIs is not just being explored for women whose tumors are HR+/HER2+ but is being tested as an alternative treatment for all HR+ breast cancer [34]. The ARNO 95 study of 2007 compared the use of aromatase inhibitor anastrozole to tamoxifen treatment in 979 postmenopausal women who had already received two years of adjuvant tamoxifen therapy for HR+ breast cancer [35]. The main purpose of this study was to compare disease-free survival among patients who switched from tamoxifen to anastrozole against those who continued with tamoxifen treatment for an additional three years. The results indicated that switching to anastozole significantly decreased the risk of cancer recurrence and improved overall survival when compared to continued tamoxifen. Those women who switched treatment also experienced a reduction in adverse side effects compared with tamoxifen. These results have changed the view of a 5-year tamoxifen treatment course as an optimal adjuvant therapy for HR+ breast cancer [32, 35]. Therefore, the use of AIs appears to be a superior form of endocrine treatment for both HR+ and HR+/HER2+ disease, showing both a better response rate and more infrequent disease recurrence.

The establishment of HER2 status in breast tumors has not only been found to predict responsiveness to the monoclonal antibody treatment, trastuzumab, but evidence now points to HER2 overexpression or amplification as a predictive marker for response to certain forms of adjuvant chemotherapy. The 1994 CALGB 8869 study was the first to draw the connection between HER2 and anthracycline treatment describing HER2 status as a potential molecular marker of patients who will respond well to high-dose chemotherapy [36]. Many different kinds of chemotherapy drugs and regimens such as anthracyclines, taxanes, cyclophosphamide, capecitabine, fluorouracil, vinorelbine, and gemcitabine are used in the treatment of breast cancer. Currently, most studies use a CEF (cyclophosphamide, epirubicine, and fluorouracil) anthracycline-based chemotherapy treatment versus CMF (cyclophosphamide, methotrexate, fluorouracil) regimens [37–40]. Earlier studies, testing the efficacy of different chemotherapy regimens in node positive breast cancer, found CEF treatment to be more efficacious in reducing relapse and overall survival (18%) when compared to CMF treatment [39, 41, 42]. These findings are attributed to this increased response to anthracycline-based CEF treatment to the overexpression or amplification of HER2 in these breast cancer tumors. The results of several recent studies agree that increased responsiveness to anthracycline treatment seems to be specific to HER2+ breast tumors [37, 39, 40, 43].

In 2004, Petit et al. [38] tested the predictive value of five different biologic factors in patients with breast cancer receiving neoadjuvant, anthracycline-based chemotherapy. One of those factors tested the influence of HER2 and topoisomerase II alpha (TopoIIα) amplification in response to anthracycline chemotherapy. TopoIIα is an enzyme with its gene located very close to HER2/neu on chromosome 17. It has been found that when HER2 is amplified, frequently TopoIIα is coamplified. This may be relevant as TopoIIα has been found to be one of the molecular targets of anthracyclines. Interestingly, Petit et al. [38] does support data suggesting coamplification of HER2 and TopoIIα but found no correlation between HER2/TopoIIα amplification and response to anthracycline chemotherapy. In contrast, the 2006 Scandinavian Breast Group Trial 9401, using the anthracycline epirubicin, found that TopoIIα amplification reduced the relapse rate in patients treated with tailored and dose-increased epirubicin treatment [40]. In addition, HER2 amplification was found to be predictive of short, relapse free, and overall survival but was not found to have a direct association with treatment assignment. Therefore, this study provides evidence that directly supports TopoIIα as a useful marker for determining the effectiveness of anthracycline treatment.

This recent data suggests that the amplification of HER2 indirectly predicts anthracycline chemotherapy response due to coamplification of TopoIIα. However, further investigation into the role of TopoIIα amplification in anthracycline treatment response is necessary to definitively establish its direct contribution. To date, current data supports the treatment of HER2+ breast cancer with high-dose anthracycline treatment like CEF, whereas HER2-disease may benefit more from the less-toxic CMF chemotherapy regimen.

Naturally occurring phytoestrogens (or phytochemicals) have also been identified in having a chemopreventive role in breast cancer. Phytoestrogens are polyphenols that are divided into several primary groups: the isoflavones (genistein, daidzein, biochanin A), the lignans (enterolactone, enterodiol), the coumestans (coumestrol) and the stilbenes (resveratrol). These polyphenols have a strong structural homology to 17β-estradiol and occur naturally in soybeans and soybean-based foods. Metabolic activation of isoflavones occurs locally within a target tissue, such as breast tissue, and can be detected through the production of metabolites [44]. Preliminary studies assessing the effects of isoflavones on steroid hormone levels have found that women with a diet rich in soy food products have a significantly lower concentration of serum 17β-estradiol [45, 46]. This is said to occur through the ability of phytochemicals to suppress the activity of aromatase enzymes that convert androgens to estrogens [47]. This is of importance because a lower lifetime exposure to estrogen has been correlated with a reduction in breast cancer risk. One study has also found a 40% increase in urinary excretion of anticancer metabolite 2-hydroxyoestrone following the consumption of 113–202 mg of isoflavones in premenopausal women [48]. Therefore, research suggests that isoflavones afford chemoprotection by suppressing steroid hormone biosynthesis and promoting the breakdown of estradiol into anticancer metabolites.

Studies using rodent models have evaluated the effects of phytochemicals on the initiation and growth of pre-existing tumors. One study testing the effect of genistein on transgenic mice overexpressing the HER2 gene found that genistein delayed mammary tumor onset. Recently, these researchers performed a study to determine the effect of genistein on an ERα-/HER2+ cell line called BT-474 cells. They demonstrated that at high doses genistein acts as a potent tyrosine kinase inhibitor blocking HER2 phosphorylation/activation and thereby inhibiting signaling through the MAPK pathway. In addition, in ERα-cells, genistein was also found to inhibit HER2 total protein expression by ~50%. Therefore, this study concluded that genistein at a dosage high enough to inhibit tyrosine kinase activity could also inhibit HER2 promoter regions in the absence of ERα and thus decrease HER2 expression through inhibiting it transcriptionally. Through different experimental trials, these researchers concluded that this inhibitory effect on

HER2 transcription and total protein expression only occurs in the absence of ER alpha [49]. Therefore, genistein has been identified as an effective treatment option for HER2+/ERα-breast cancer for its ability to inhibit cell growth through these mechanisms.

A 2005 study assessing the effect of the stilbene resveratrol on spontaneously occurring HER2+ mammary tumors of transgenic mice further supports the utility of phytochemicals as a potential treatment. Results of this study showed that when resveratrol supplementation was given, these mice exhibited a delayed spontaneous mammary tumor development ($P < 0.001$) with a reduction in the mean number and size of mammary tumors ($P < 0.0001$). Resveratrol treatment was also associated with downregulation of HER2/neu gene expression and increased apoptosis of HER2/neu overexpressing mammary tumor cells [50]. Together, these studies suggest that genistein and resveratrol exert antitumor effects on HER2+ mammary tumors through their ability to inhibit HER2 activation and protein expression, making supplementation with these natural and inexpensive phytochemicals, a potential adjuvant for HER2+ breast cancer.

Superior efficacy of anti-HER2 combination therapy in the treatment of HER2-positive metastatic breast cancer

Knowledge of the biologic characteristics of invasive breast cancer is critical in determining an optimal treatment course. A large number of patients with breast cancer are initially subjected to neoadjuvant or adjuvant chemotherapy, due to its proven effectiveness as a single agent therapy in women with metastatic disease. A specific cohort of patients whose tumors overexpress or amplify HER2 benefit greatly from trastuzumab treatment as demonstrated by the significant increase in overall survival of those who receive such treatment [20–23]. In addition, patients who demonstrate coexpression of HER2 and HRs (ER or PR), i.e. HER2+/HR+ tumors derive a greater clinical benefit from single agent endocrine therapy [33, 35]. However, the development of resistance to both single agent trastuzumab and endocrine therapy, such as tamoxifen, has significantly complicated the treatment of qualified patients with these forms of monotherapy. Therefore, cancer research has directed its inquiry toward the study of anti-HER2 combination therapies significantly over the past decade. This focus is justified by findings that have implicated the role of HER2 in conferring this resistance.

Combination chemo/anti-HER2 therapies have been proven to be most successful in improving partial and complete responses in most HER2+ disease compared to chemotherapy alone [51, 52]. However, the determination of which anti-HER2 therapy is most valuable in halting disease progression is still under investigation. A large body of research supports the use of trastuzumab in conjunction with anthracycline, taxane, or platinum salt-based chemotherapy treatment [52, 53]. A 2001 multinational phase III randomized trial found that combining trastuzumab with chemotherapy extended the time to disease progression (TTP; median 7.4 vs. 4.6 months; $P < 0.001$), increased the rate of objective (measurable) response (ORR; 50 vs. 32%; $P < 0.001$), and lengthened the duration of response (DR; median 9.1 vs. 6.1 months; $P < 0.001$) when compared to chemotherapy alone [52]. Moreover, this combination therapy was also associated with a lower rate of death at 1 year (22 vs. 33%; $P = 0.046$) and a 20% reduction in the risk of death.

In support of this study, a comparison of two pivotal trials, one testing the effect of standard chemotherapy and the other testing the effect of chemo/trastuzumab combination therapy, showed that the addition of trastuzumab significantly increased TTP, ORR, DR, and time to treatment failure (TTF) in HER2+ metastatic patients [51]. It is of importance to note that chemo/trastuzumab combination therapy significantly improved overall survival in comparison with chemotherapy alone. In fact, in those individuals who were HER2 (3+), as determined through IHC, addition of trastuzumab demonstrated a 45% increase in overall survival after 35 months follow-up. Therefore, these studies support the use of chemo/trastuzumab combination therapy as a more effective treatment then chemotherapy alone for HER2+ invasive breast cancer.

More recent studies substantiate the use of particular forms of chemotherapy in combination with trastuzumab. Specifically, these studies endorse the use of taxanes and platinum salts as more effective in improving pathological complete response (pCR) as well as overall response rates. These forms of chemotherapy have undergone clinical trial because preclinical data suggests that the combination of taxanes, such as docetaxel, and platinum salts act in synergy with trastuzumab [54]. When combined with trastuzumab, the cytotoxic effects of docetaxel and platinum salts, cisplatin and carboplatin, were additive. The exact mechanism of interaction between these drugs is still unclear, but the data suggest that activation of the apoptotic pathway is increased [55].

Data from two phase II clinical trials, UCLA-ORN and BCIRG, which evaluated docetaxel with either cisplatin or carboplatin in combination with trastuzumab, exhibited the longest times to progression ever reported for patients with HER2+ metastatic breast cancer (BCIRG-9.9 and UCLA-ORN 12.7 months) [55]. Overall response rates in this patient population were also reported to be increased (BCIRG-79% and UCLA-ORN-58%). In 2006, Hurley and colleagues, also testing the efficacy of docetaxel, cisplatin, and trastuzumab combination therapy in the HER2+ population, reported successful values for progression-free

survival (PFS) and overall survival (OS) [54]. At a median follow-up time of 43 months, the 4-year PFS rate was at 81% (95% CI, 64–90%) with an OS rate of 86% (95% CI, 71–94%). Together, these clinical trials support docetaxel, cisplatin or carboplatin, and trastuzumab combination therapy to be clinically active in HER2+ breast tumors as well as advocate this therapy's ability to significantly increase patient's survival rates.

Unfortunately, some women with HER2+ breast cancer never respond to trastuzumab and are said to have primary/de-novo resistance. Furthermore, some may initially respond and develop secondary resistance usually within 1 year of treatment. The mechanism of this resistance is uncertain. One theory suggests that steric hinderance of the receptor–antibody interaction may be the cause. The glycoprotein MUC4 can act as a ligand for HER2 and is known for its ability to inhibit immune recognition of cancer cells and thus promoting tumor progression, metastasis, and suppression of apoptosis. Overexpression of MUC4 obscures HER2 binding sites for trastuzumab thus hindering the interaction between antibody and therapeutic target [56]. This correlation between high MUC4 levels and decreased trastuzumab binding capacity has been proposed as a potential mode of trastuzumab resistance [56].

The use of the dual tyrosine kinase inhibitor lapatinib (Tykerb) as an alternative mode of HER2 inactivation has been successful in treating HER2 patients resistant to trastuzumab [57]. Since lapatinib binds to the intracellular domain of EGFR and HER2, its activity does not require access to the entire receptor [57]. Therefore, tyrosine kinase inhibitors provide another alternative method of blocking HER2 transduction. Recent clinical trials have tested the usefulness of anti-HER2 drug, lapatinib, in women who were previously unresponsive to monoclonal antibodies. Two trials in particular tested the combination of lapatinib with the chemotherapy drug capecitabine, against capecitabine alone, in women with advanced metastatic breast cancer that had progressed after treatment regimens that included an anthracycline, a taxane, and trastuzumab. Both studies concluded that lapatinib/capecitabine combination therapy demonstrated superior efficacy to capecitabine alone in women with HER2+ metastatic cancer that had previously been unresponsive to trastuzumab [57]. Notably, in the follow-up trial, these women saw significant gains in the median TTP from 4.3 to 6.2 months. Therefore, in the case of women with HER2+ breast cancer that is resistant to trastuzumab, tyrosine kinase inhibitors like lapatinib have provided a successful alternative treatment option.

A subpopulation of women with breast cancer tumors that are HER2+/HR+ have been found to benefit from adjuvant endocrine therapy. However, recent findings associating the overexpression of HER2 with the problem of primary and secondary resistance to endocrine therapy have directed research toward the investigation of anti-HER2/endocrine combination therapy. This combination therapy could become the standard of treatment if clinical results prove efficacious for those with HER2+/HR+ breast cancer. In 2006, a phase II clinical study enrolled 33 patients to be treated with the aromatase inhibitor letrozole in combination with trastuzumab [58]. Twenty-five of these patients (82%) had received prior tamoxifen treatment with 16 of these cases (48%) experiencing recurrence while taking tamoxifen. The vast majority of these cases (73%) had visceral metastatic sites. In addition, 82% of these patients were confirmed as HER2+ (FISH+ and or IHC3+) breast tumors. Combination therapy with letrozole and trastuzumab yielded an overall response rate of 26% and a clinical benefit rate of 52% [58]. These responses were reported to last at least one year in 25% of the patients, and combining these two therapies was shown to be well tolerated with a low incidence of toxic side effects.

The issue of endocrine/anti-HER2 combined therapy was also assessed in the randomized phase III TAnDEM study [2, 35]. This study enrolled 208 patients with HER2+/HR+ postmenopausal metastatic breast cancer to receive first-line anastrozole/trastuzumab treatment or anastrozole monotherapy. The majority of enrolled patients had received prior endocrine therapy. Anastrozole/trastuzumab combination therapy was found to significantly improve progression-free survival compared with anastrozole alone [2, 35]. Combination therapy also increased overall survival compared to the monotherapy group (28.5 vs. 23.9 months). In summary, the results of these clinical trials exhibit a trend toward prolonged overall survival and an increase in responsiveness to combination therapy compared to endocrine monotherapy.

One drawback to the use of anti-HER2 combination therapies is the increased incidence of cardiotoxicity, especially in patients receiving the combination of trastuzumab plus chemotherapy. For example, in the aforementioned 2001 multinational phase III randomized trial combining trastuzumab plus chemotherapy, patients experienced an extremely high rate of cardiac dysfunction (occurring in 27% of the group given an anthracycline, cyclophosphamide, and trastuzumab) [52]. Analyses of phase II and III clinical trials demonstrated that cardiotoxicity associated with trastuzumab is higher when administered with anthracyclines and may actually be an exacerbation of anthracycline-induced cardiotoxicity. Although the mechanism by which trastuzumab increases cardiotoxicity induced by anthracyclines is not yet understood, it should be mentioned that the cardiotoxicity experienced by patients undergoing this combination therapy is largely reversible with appropriate medical treatment [20, 24].

Conclusions

Treatment of HR+ breast cancer has become complicated due to the development of primary and secondary resistance to endocrine therapy. This problem is of great importance considering that over half of all breast cancers test HR positive. HER2 has been implicated in conferring such resistance due to studies that have shown a positive correlation between HER2 overexpression and increased tamoxifen resistance. Tumors testing positive for the HER2 receptor account for 25–30% of all types of breast cancer. HER2 positive breast cancer has been associated with a fast progression rate and a poor prognosis. Research investigating the mechanism by which ER+ tumors establish resistance to antiestrogen therapy has found that the ER has nongenomic activities associated with activation of EGFR and HER2. This crosstalk has been shown to enhance the growth promoting effects of estrogen, the ligand for the ER, which allows the breast cancer to progress.

The MAPK pathway has been shown to play a pivotal role in the activation of the ER by EGFR and HER2. This activation has been found to promote the expression of genes that favor cell proliferation and survival. In addition, ERK has also been found to phosphorylate and activate the ER coactivator A1B1. Studies have found that higher levels of the coactivator A1B1 amplify the agonist activities of estrogen-bound tamoxifen. This may explain why breast cancer patients displaying high HER2/neu and A1B1 levels exhibit tumor progression and poor recovery.

The development of different forms of single agent biologic therapies targeting the HER2 receptor has been successful in slowing and even halting tumor progression. The development of the recombinant humanized monoclonal antibody, trastuzumab, has been successful in eliciting treatment response in HER2+ patients previously unresponsive to chemotherapy. Trastuzumab treatment was also better tolerated among these patients, producing fewer adverse side effects. While some patients exhibit resistance to trastuzumab treatment, alternative treatment with tyrosine kinase inhibitors such as lapatinib, have been proven to be clinically active and tolerable in HER2+ breast cancer. The mechanism of trastuzumab resistance is uncertain, although one theory suggests that the overexpression of glycoprotein MUC4 blocks HER2 binding sites that sterically hinder the binding of trastuzumab.

Patients with HR+/HER2+ breast cancer have shown a better response to single agent aromatase inhibitors than estrogen receptor modulators such as tamoxifen. As HER1 and HER2 signaling has been found to be dependent upon estrogen, estrogen deprivation through the use of AIs has been shown to be more successful in halting tumor progression in these patients. The determination of HER2 status in patients with breast cancer has also been found to be a predictive marker for response to specific forms of single agent chemotherapy. Recent studies agree that HER2+ breast tumors show increased responsiveness to anthracycline-based chemotherapy regimens, which has been attributed to the coamplification of TopoIIα with HER2. As TopoIIα is one of the molecular targets of anthracyclines, it may prove a useful marker for determining proper anthracycline dosage.

The development of resistance to various forms of monotherapy has necessitated the exploration of various forms of anti-HER2 combination therapies. The success of different forms of combination therapy is dependent upon the biologic characteristics of each patient's breast cancer disease. The use of combination therapies appropriate to the biologic characteristics of a particular patient's presentation of HER2+ breast cancer is strongly correlated with an increase in disease-free survival as well as overall survival rates. Therefore, determination of tumor HER2, HR, TopoIIα, MUC4 and A1B1 levels is critical in the selection of the appropriate treatment regimen. Results from various studies have shown that combination therapies can provide increased efficacy and often superior alternatives to single agent chemotherapy, and therefore should become the standard of treatment for HER2+ breast cancer.

Future studies

More research further exploring treatments with fewer adverse side effects such as trastuzumab, HER2/neu vaccinations, and phytochemical treatment is warranted and highly relevant in HER2 breast cancer research. Further inquiry directed toward eliminating the overexpression of MUC4, possibly through targeting MUC4 promoter regions in order to decrease MUC4 expression, may help reveal the exact mechanism of trastuzumab resistance in HER2+ breast cancer. This research could make trastuzumab monotherapy an effective alternative to more harsh combination treatments involving chemotherapy. In addition, long-term controlled clinical trials are highly necessary in determining the effectiveness of HER2/neu peptide vaccinations and phytochemical supplementation in slowing or eliminating tumor progression in HER2+ breast cancer patients. Studies evaluating the utility of resveratrol in halting tumor progression recognize that much more research is needed in order to determine the exact binding proteins and pathways involved in resveratrol action. Evidence proving these treatments to be effective in halting disease progression in the long-term would provide HER2 patients with treatment options that are successful yet less harmful to the patient's overall health.

Currently, new anti-HER2 drugs and drug combinations have been the focus of ongoing studies. Genetech has

recently been developing another monoclonal antibody against HER2, pertuzumab (Omnitarg, 2C4), which binds to the dimerization domain of HER2 inhibiting its heterodimerization with other HER family members [2]. Researchers have been studying the efficacy of combining pertuzumab with trastuzumab and have found that these two drugs seem to act synergistically to increase apoptosis and thus inhibit the survival of BT474 breast cancer cells. Results have indicated that combining these drugs is more effective in halting disease progression than either drug alone. In addition, combining pertuzumab with other anti-HER2 drugs, such as tyrosine kinase inhibitors, is of interest in treating cancers that not only overexpress HER2 but also EGFR [59]. One study treated patients with advanced HER2+ breast cancer, which had progressed after initial trastuzumab treatment, with the orally administered tyrosine kinase inhibitor, neratinib (HKI-272) combined with trastuzumab. Findings indicated that neratinib in combination with trastuzumab was well tolerated and clinically active in advanced HER2+ breast cancer [60].

Most recently, a unique form of combination therapy involving the drug trastuzumab-DM1 (TDM1) is being studied as a less-toxic method of delivering chemotherapy directly to cancerous tissue. This therapy combines trastuzumab with the chemotherapy agent, maytansine (DM1) an inhibitor of tubulin polymerization, and has been termed immunoconjugate therapy. This type of therapy utilizes antibodies as a mode of getting cytotoxic agents specifically to tumors expressing the corresponding antigens. Trastuzumab-DM1 is selective for HER2 overexpressing tumors and has thus far proven to be an active agent, even in trastuzumab refractory tumors. Currently, trastuzumab-DM1 is being developed clinically for its application in treating HER2 metastatic disease [61]. Additional research targeting heat shock protein 90, which is pivotal in the maturation of oncogenic protein HER2, has shown to be beneficial in promoting the ubiquitinization and subsequent degradation of HER2 [2, 62]. Other research has sought to inhibit mTOR, a central regulator of the G1 cell cycle. Results of one study showed that mTOR inhibitor CCI-779 effectively inhibited proliferation of HER2/neu expressing BT474 and SKBR-3 cells, thus making it yet another potential target in the regulation of HER2+ breast cancer [63].

Lastly, it has been found that patients with HER2+ metastatic breast cancer are at a significantly higher risk for the development of brain metastases, in fact, nearly one-third of breast cancer patients with tumors overexpressing HER2 develop brain metastases, especially those who had been previously treated with trastuzumab. Even though trastuzumab effectively treated patients with lung and liver metastases, these patients still went onto develop brain cancer. Due to these findings, researchers have concluded that trastuzumab may be effective at inhibiting HER2 in the liver, lungs, and breast but is too large to cross the blood–brain barrier (BBB) and reach a concentration adequate for HER2 inhibition. Therefore, lapatinib, a small molecule tyrosine kinase inhibitor, is being used in recent clinical trials due to its ability to cross the BBB and in turn block HER2 in the brain as well as other HER2 metastatic sites in the body. Current results of clinical trials indicate that lapatinib is well tolerated and is clinically active in treating CNS brain metastasis [64].

References

1. Jemal A, Siegel R, Ward E et al (2008) Cancer statistics, 2008. Cancer J Clin 58:71–96
2. Prat A, Baselga J (2008) The role of hormone therapy in the management of the hormonal-receptor-positive breast cancer with co-expression of HER2. Nat Clin Pract Oncol 5(9):531–542
3. Bartlett JMS, Going JJ, Mallon EA et al (2001) Evaluating HER2 amplification and expression in breast cancer. J Pathol 195:422–428
4. Lipton A, Ali SM, Leitzel K et al (2002) Elevated Serum HER-2/neu level predicts decreased response to hormone therapy in metastatic breast cancer. J Clin Oncol 20(6):1467–1472
5. Chang JC (2007) HER2 inhibition: from discovery to clinical practice. Clin Cancer Res 13:1–3
6. Rubin I, Yarden Y (2001) The basic biology of HER2. Ann Oncol 12(Suppl. 1):S3–S8
7. Cooke T (2000) What is HER2? Eur J Oncol Nurs 4(Suppl. 1):2–9
8. Ross JS, Fletcher JA, Linette GP et al (2003) The HER-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. Oncol 8:307–325
9. Liu Y, El-Ashry D, Chen D et al (1995) MCF-7 breast cancer cells overexpressing transfected c-erbB-2 have an in vitro growth advantage in estrogen-depleted conditions and reduced estrogen-dependence and tamoxifen-sensitivity in vivo. Breast Cancer Res Treat 34:97–117
10. Kurokawa H, Lenferink AEG, Simpson JF et al (2000) Inhibition of HER2/neu (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. Cancer Res 60:5887–5894
11. Osborne KC, Bardou V, Hopp TA (2003) Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. J Natl Cancer Inst 95(5):353–361
12. Shou J, Massarweh S, Osborne KC et al (2004) Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. J Natl Cancer Inst 96(12):926–935
13. Osborne KC (1998) Tamoxifen in the treatment of breast cancer. N Engl J Med 339(22):1609–1618
14. Schiff R, Massarweh SA, Shou J et al (2004) Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. Clin Cancer Res 10:331s–336s
15. Smith CL, Nawaz Z, O'Malley BW (1997) Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11(6):657–666
16. Razandi M, Pedram A, Park ST, Levin ER (2003) Proximal events in signaling by plasma membrane estrogen receptors. J Biol Chem 278(4):2701–2712
17. Font De Mora J, Brown M (2000) A1B1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor. Mol Cell Biol 20(14):5041–5047

18. Hendriks BS, Orr G, Wells A, Wiley HS, Lauffenburger DA (2005) Parsing ERK activation reveal quantitatively equivalent contributions from EGFR and HER2 in human mammary epithelial cells. J Biol Chem 280:6157–6169
19. Cobleigh MA, Vogel CL, Tripathy D et al (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17(9):2639–2648
20. Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 353(16):1659–1672
21. Vogel CL, Cobleigh MA, Tripathy D et al (2002) Efficacy and Safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20(3):719–726
22. Vogel C, Cobleigh MA, Tripathy D et al (2001) First-line, single-agent Herceptin (trastuzumab) in metastatic breast cancer: a preliminary report. Eur J Cancer 37:S25–S29
23. Vogel CL, Cobleigh MA, Tripathy D et al (2001) First-line herceptin monotherapy in metastatic breast cancer. Oncology 61(Suppl. 2):37–42
24. Suter TM, Cook-Bruns N, Barton C (2004) Cardiotoxicity associated with trastuzumab (Herceptin) therapy in the treatment of metastatic breast cancer. Breast 13:173–183
25. Burstein HJ, Storniolo AM, Franco S et al (2008) A phase II study of lapatinib monotherapy in chemotherapy-refractory HER2-positive and HER2-negative advanced or metastatic breast cancer. Ann Oncol 19:1068–1074
26. Nahta R, Yuan LXH, Du Y, Esteva FJ (2007) Lapatinib induces apoptosis in trastuzumab-resistant breast cancer cells: effects on insulin-like growth factor I signaling. Mol Cancer Ther 6(2):667–674
27. Baselga J, Albanell J, Ruiz A et al (2005) Phase II and tumor pharmacodynamic study of gefitinib in patients with advanced breast cancer. J Clin Oncol 23:5323–5333
28. Gomez HL, Doval DC, Chavez MA et al (2008) Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer. J Clin Oncol 26(18):2999–3005
29. Avigan D, Vasir B, Gong J et al (2004) Fusion cell vaccination of patients with metastatic breast and renal cancer induces immunological and clinical responses. Clin Cancer Res 10:4699–4708
30. Disis ML, Gooley TA, Rinn K et al (2002) Generation of T-Cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. J Clin Oncol 20(11):2624–2632
31. Disis ML, Schiffman K, Guthrie K et al (2004) Effect of dose on immune response in patients vaccinated with an HER-2/neu intracellular domain protein-based vaccine. J Clin Oncol 22(10):1916–1925
32. Jakesz R, Jonat W, Gnant M et al (2005) Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ANCSG trial 8 and ARNO 95 trial. Lancet 366:455–462
33. Ellis MJ, Coop A, Singh B et al (2001) Letrozole is more effective Neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or Erb-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol 19(18):3808–3816
34. Mouridsen H, Gershanovich M, Sun Y et al (2001) Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the international letrozole breast cancer group. J Clin Oncol 19(10):2596–2606
35. Kaufmann M, Jonat W, Hilfrich J et al (2007) Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: the ARNO 95 study. J Clin Oncol 25(19):2664–2669
36. Muss HB, Thor AD, Berry DA et al (1994) c-erbB-2 Expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med 330(18):1260–1266
37. Paik S, Bryant J, Tan-Chiu E et al (2000) HER2 and choice of adjuvant chemotherapy for invasive breast cancer: national surgical adjuvant breast and bowel project protocol B-15. J Natl Cancer Inst 92(24):1991–1998
38. Petit T, Wilt M, Velten M et al (2004) Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 40:205–211
39. Pritchard KI, Shepherd LE, O'Malley FP et al (2006) HER2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med 354(20):2103–2111
40. Tanner M, Isola J, Wiklund T et al (2006) Topoisomerase IIα gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu-amplified breast cancer: Scandinavian breast group trial 9401. J Clin Oncol 24(16):2428–2436
41. Levine MN, Bramhall VH, Pritchard KI et al (1998) A randomized trial of intensive cyclophosphamide, epirubicin, and fluorouracil chemotherapy compared with cyclophosphamide, methrotrexate, and fluorouracil in premenopausal women with node positive breast cancer. J Clin Oncol 16:2651–2658
42. Levine MN, Pritchard KI, Bramhall VH et al (2005) A randomized trial comparing cyclophosphamide, epirubicin, and fluorouracil with cyclophosphamide, methrotrexate, and fluorouracil in premenopausal women with node positive breast cancer: update of National Cancer Institute of Canada Clinical Trials Group Trial MA5. J Clin Oncol 23:5166–5170
43. Gennari A, Sormani MP, Pronzato P et al (2008) HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials. J Natl Cancer Inst 100(1):14–20
44. Limer JL, Speirs V (2004) Phytoestrogens and breast cancer chemoprevention. Breast Cancer Res 6(3):119–127
45. Lu L-J, Anderson KE, Grady JJ, Kohen F, Nagamani M (2000) Decreased ovarian hormones during a soya diet: implications for breast cancer prevention. Cancer Res 60:4112–4121
46. Kumar NB, Cantor A, Allen K, Riccardi D, Cox CE (2002) The specific role of isoflavones on estrogen metabolism in premenopausal women. Cancer 94:1166–1174
47. Grube BJ, Eng ET, Kao Y-C, Kwon A, Chen S (2001) White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. J Nutr 131:3288–3293
48. Lu L-J W, Cree M, Josyula S, Nagamani M, Grady JJ, Anderson KE (2000) Increased urinary excretion of 2-hydroxyestrone but not 16α-hydroxyestrone in premenopausal women during a soya diet containing isoflavones. Cancer Res 60:1299–1305
49. Sakla MS, Shenouda NS, Ansell PJ, MacDonald RS, Lubahn DD (2007) Genistein affects HER2 protein concentration, activation, and promoter regulation in BT-474 human breast cancer cells. Endocrine 32:69–78
50. Provinciali M, Re F, Donnini A et al (2005) Effect of resveratrol on the development of spontaneous mammary tumors in HER-2/*neu* transgenic mice. Int J Cancer 115:36–45
51. Baselga J (2001) Herceptin Alone or in Combination with Chemotherapy in the Treatment of HER2-Positive Metastatic Breast Cancer: Pivotal Trials. Oncology 61(suppl 2):14–21
52. Slamon DJ, Leyland-Jones B, Shak S et al (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metatstatic breast cancer that overexpresses HER2. N Engl J Med 334(11):783–792

53. Buzdar AU, Ibrahim NK, Francis D et al (2005) Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 23:3676–3685
54. Hurley J, Doliny P, Reis I et al (2006) Docetaxel, cisplatin, and trastuzumab as primary systemic therapy for human epidermal growth factor receptor 2-positive locally advanced breast cancer. J Clin Oncol 24(12):1831–1838
55. Pegram MD, Pienkowski T, Northfelt DW et al (2004) Results of two open-label, multicenter phase II studies of docetaxel, platinum salts, and trastuzumab in HER2-positive advanced breast cancer. J Natl Cancer Inst 96(10):759–769
56. Nahta R, Esteva FJ (2006) Molecular mechanisms of trastuzumab resistance. Breast Cancer Res 8(6):667–674
57. Cameron D, Casey M, Press M et al (2008) A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 112:533–543
58. Marcom PK, Isaacs C, Harris L et al (2007) The combination of letrozole and trastuzumab as first or second-line biological therapy produces durable responses in a subset of HER2 positive and ER positive advanced breast cancers. Breast Cancer Res Treat 102:43–49
59. Nahta R, Hung M-C, Esteva FJ (2004) The HER2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res 64:2343–2346
60. Swaby R, Blackwell K, Jiang Z et al (2009) Neratinib in combination with trastuzumab for the treatment of advanced breast cancer: a phase I/II study. J Clin Oncol 27(15S):1004
61. Lewis Philips GD, Li G, Dugger DL (2008) Targeting HER2 positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res 68:9280
62. Kamal A, Thao L, Sensintaffer J et al (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425:407–410
63. Yu K, Toral-Barza L, Discafani C et al (2001) mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. Endocr Relat Cancer 8:249–258
64. Moy B, Goss PE (2006) Lapatinib: current status and future directions in breast cancer. Oncol 11(10):1047–1057