The Biological Therapy of Breast Cancer

Molecular Targets and Monoclonal Antibodies
HER2 and Herceptin

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Investigational trials in breast cancer

Introduction

Breast cancer is a disease of global medical, social, and economic impact. For the year 2003 there will be an estimated 212,600 new cases in the U.S. with 40,200 deaths [1]. Breast cancer is the second leading cause of cancer death in women despite the fact that overall death rates from this disease have declined worldwide since 1998.

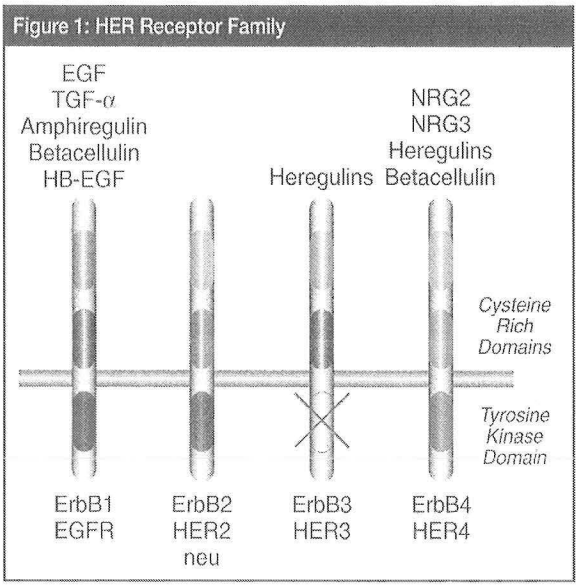
Breast cancer treatment has evolved significantly over the past several decades. Adjuvant chemo and hormonal therapies have reduced recurrence and death rates and reflected in the Early Breast Cancer Trials-Collaborative Group Overviews [2]. From the 1970's throughout the 1990's multiple new chemotherapy drugs and combination regimens were developed to improve breast cancer outcomes. Generally these therapies interrupt or inhibit cell division and DNA repair or replication. Such treatments are not tumor cell specific and can cause severe toxic side effects to normal cells. The impetus has now shifted to develop therapies with greater effectiveness and lesser toxicity. To achieve this goal, new therapies must target the molecular factors and pathways controlling breast cancer cell growth and metastases. In 1987 Dennis Slamon described a distinct subset of breast cancer patients whose tumor cells had HER2 gene amplification and its protein overexpression on the cell membranes. Amplification was a significant predictor of overall survival and time to relapse in these patients [3]. This observation and much subsequent work have spotlighted the role of the EGF family of receptors, especially HER2, in the biologic behavior and pathogenesis of human breast cancer.

Research to develop anti HER2 therapy resulted in the development of Trastuzumab (Herceptin®), a humanized murine monoclonal antibody directed against the HER2 extracellular domain. In 1998 the FDA approved Herceptin for the treatment of HER2 overexpressing metastatic breast cancer. Since then, a flurry of research has been directed at understanding the biology and clinical uses of this targeted molecule.

The Epidermal Growth Factor Receptor Family

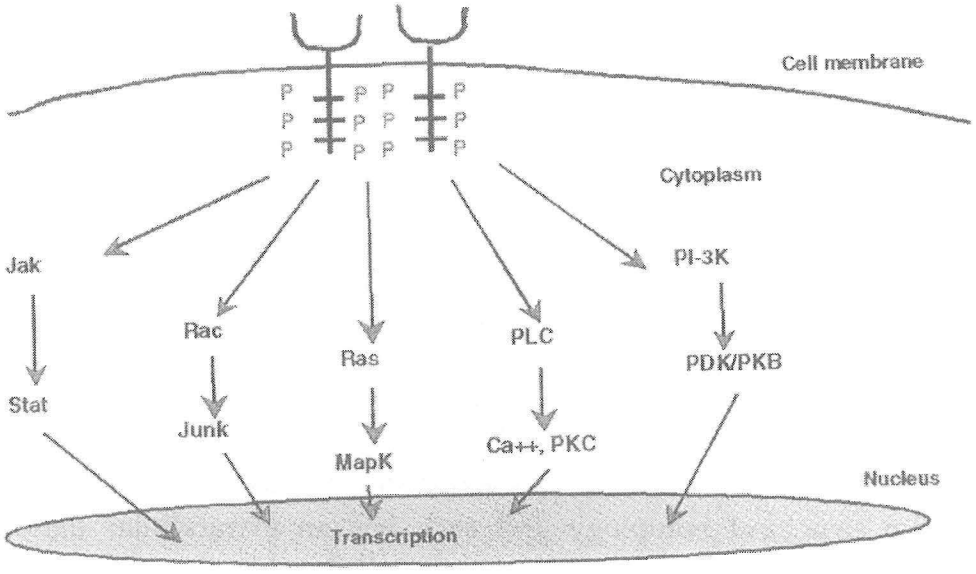
The human epidermal growth factor receptor (EGFR) family consists of four members designated as HER1 (EGFR), HER2, HER3 and HER4. These four membrane associated receptors share structural homology and each has an extracellular ligand binding domain, a transmembrane domain and an intracellular domain with tyrosine kinase catalytic activity. The receptors normally exist as monomers on the cell membrane but after ligand binding they dimerize.

Figure 1



Structurally dimers are either homo or heterodimers depending on the receptor partner. After ligand binding and dimerization, tyrosine phosphorylation of the kinase domain leads to activation of downstream transduction pathways and signaling through Ras, c-Src, PI3K or phospholipase C to control cell proliferation and differentiation, invasion, and migration [4,5].

Figure 2



There are six ligands for HER1 – EGF, TGF ∝, amphiregulin, betacellulin, epiregulin and Heparin binding EGF-like growth factor. HER2 has no known specific ligand. HER3 and HER4 bind neuregulins a family of peptides signaling through MAPK [6].

HER2 is the preferred partner for heterodimerization with the other three members of the EGFR family and their ligands. Heterodimers containing HER2 have higher ligand binding affinity and more signal potency than dimers without HER2. HER2 HER3 is the most potent signaling heterodimers. HER3 has an inactive kinase domain so HER3 homodimers are inactive. HER2 homodimers have constitutive activation and signaling despite lack of a ligand.

Additionally, the HER2 extracellular domain (ECD) can be proteolytically cleaved off the cell membrane and circulate in the serum. The ECD shedding results in constitutive activation of the remaining membrane associated HER2 domain (p95) leading to increased signal transduction. The HER2 cleavage mechanism is not yet characterized but is associated with a more aggressive cell phenotype [5].

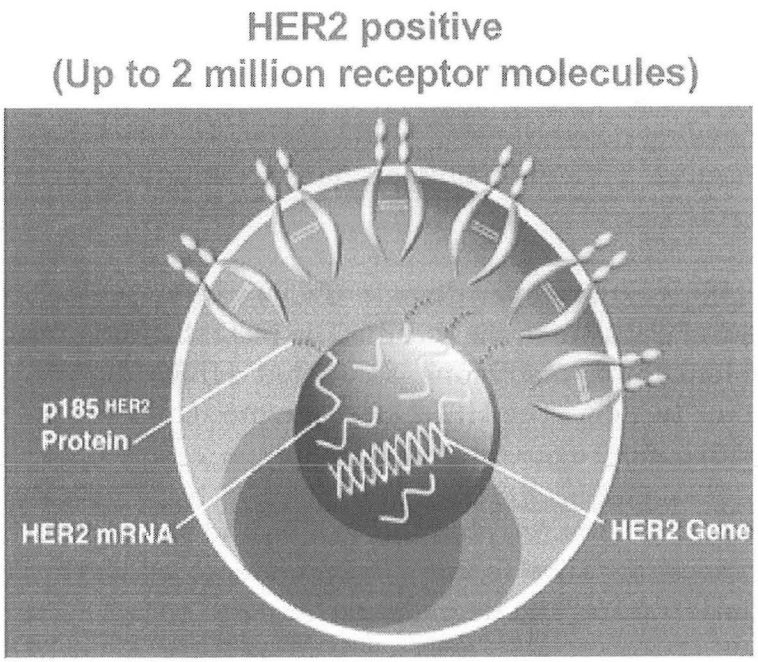
After ligand binding, dimerization, phosphorylation and signal transduction, the ligand dimer complex dissociates. The receptor may undergo endocytosis with membrane recycling or liposomal transfer and degradation [7,8].

HER2 Biology

The HER2 Receptor – Overexpression and Amplification

The HER2 protein receptor is a 185 KD transmembrane glycoprotein (p185) encoded by the HER2 gene, a proto-oncogene located on chromosome 17q21. HER2 overexpression is described in 20-25% of breast cancers with membrane protein levels 10 to 100 fold greater than normal. The protein structure of overexpressed HER2 is not mutated compared to normal. For breast cancer, gene amplification is the most common mechanism causing overexpression. The factors inducing gene amplification are not known. Gene amplification generates more than 2 normal gene copies per cell yielding increased HER2 mRNA and increased synthesis of HER2 membrane protein. In 7% of breast cancer, overexpression is not secondary to amplification but results from transcriptional or post-transcriptional dysregulation. This situation is also true in lung, bladder and esophagus cancers where overexpression is not linked to amplification.

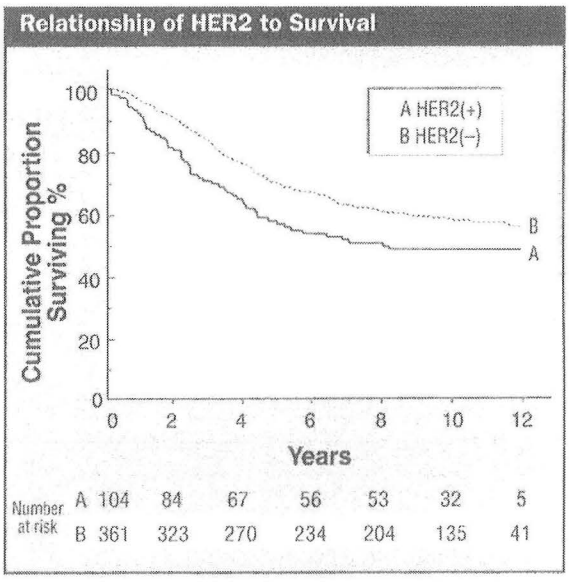
Figure 3



Relationship of HER2 and Breast Cancer

Oncogenic Transformation – lab evidence confirms that HER2 overexpression plays a pathogenic role in malignant transformation of breast cells. A range of in vitro and animal studies support a direct role of HER2 overexpression in oncogenic transformation and tumorigenesis. In breast cancer cell lines and transformed cells, HER2 gene amplification results in an aggressive phenotype with increased DNA synthesis, cell growth and metastatic potential [9-12]. This same behavior is seen clinically in HER2 overexpressing breast cancer where survival curves display a difference in long-term outcomes between HER2 positive and negative patients.

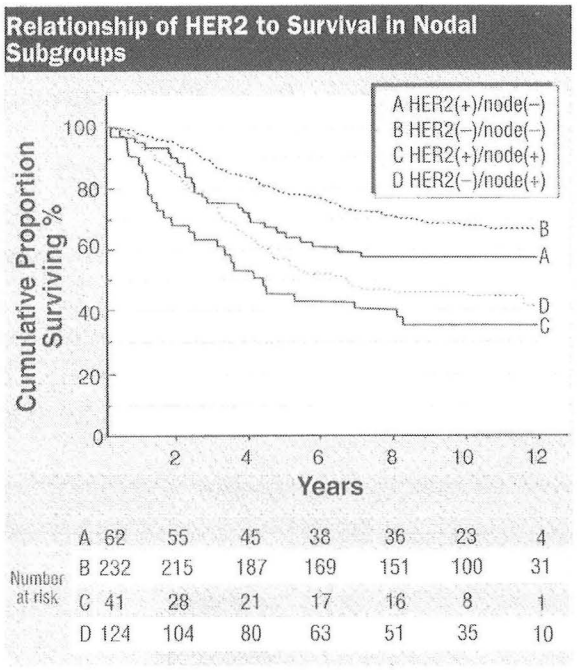
Figure 4



HER2 as a Prognostic Factor in Breast Cancer

HER2 gene amplification has independent prognostic significance to predict decreased disease-free and overall survival in multivariate analysis of node positive patients as first reported in 1987 by Slamon [3]. This observation was confirmed in a meta analysis of 47 studies of more than 15,000 patients demonstrating a positive correlation between HER2 positivity and poor prognosis [13]. This prognostic effect is reported for both node negative and node positive patients [14] and survival curves demonstrate poorer longer-term outcomes for HER2 positive patients regardless of nodal status.

Figure 5



HER2 - The Therapeutic Target

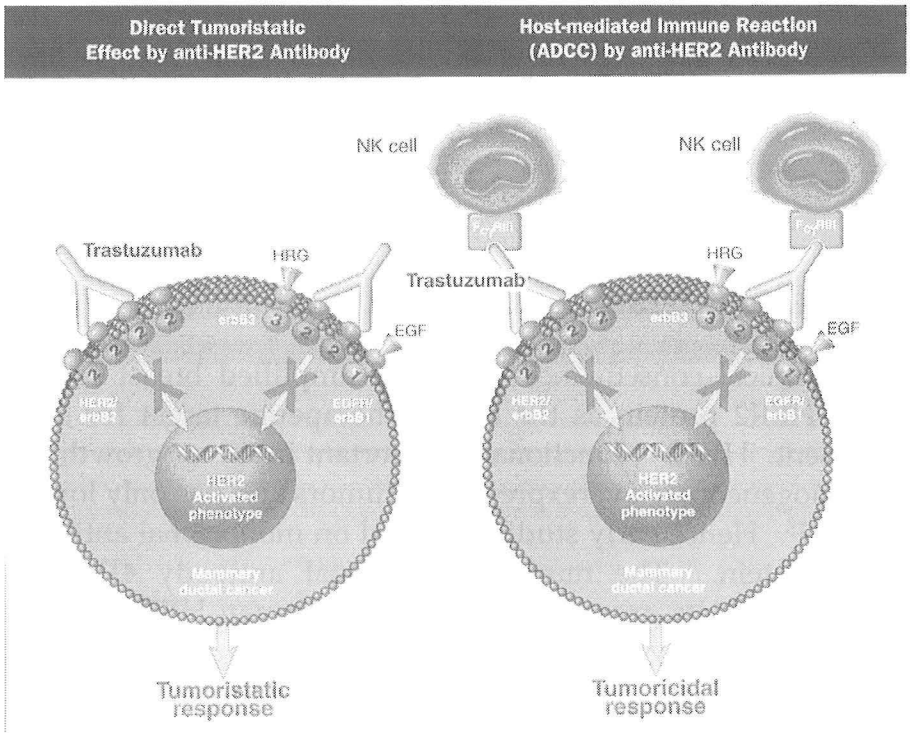
Because of the clinical consequences of HER2 amplified breast cancer, investigators spotlighted the HER2 protein as the logical therapeutic target for specific anticancer agent development. HER2 is functionally important in tumor growth, accessible on the cell surface, homogeneously overexpressed in tumors and has only low-level expression in normal tissue [15]. Hence early studies focused on monoclonal antibody development against HER2 protein. The murine monoclonal antibody 4D5 was selected for development based on its growth suppression of human HER2 xenografts in murine studies [16]. The antibody was humanized to prevent a human immunologic response (HAMA) against murine protein on repetitive administration. Using genetic engineering techniques, the antibody region of 4D5 was inserted into a human IgG framework yielding trastuzumab (Herceptin®) [17].

Mechanism of Herceptin Inhibition

Multiple proposed mechanisms explain the anti-tumor effects of Herceptin.

1. Down regulation of HER2 cell surface expression with cell phenotype transformation [18].
2. Acceleration of receptor endocytosis and lysosomal degradation. Possibly monoclonal antibody tagging the HER2 receptor recruits CbL and ubiquitination of HER2 [19,20].
3. HER2 heterodimer disruption especially the HER2 HER3 heterodimer [21].
4. Decreased downstream signaling with decreased VEGF levels and anti-angiogenic activity.
5. Decreased HER2 dimer signaling down P13K pathway and decreased HER2 HER3 signaling via MAPK.
6. Initiation of G1 arrest and p27 induction blocking G1 to S transition by blocking cyclin E CDK2 activity [22].
7. Prevention of ECD cleavage of HER2 protein thus blocking constitutive activation of p95 the remaining membrane domain [23].
8. Induction of host immune response (antibody directed cell mediated cytotoxicity - ADCC) against HER2 positive cells with human IgG bound to the membrane receptor with NK cell recruitment and release of cytotoxic lymphokines [24,25].

Figure 6



9. Interaction with chemotherapeutic agents with additive or synergistic antitumor effect due to DNA repair interference of chemo induced damage [26].

Selection of Patients for Herceptin Therapy

Tissue HER2 Evaluation

The antitumor effects of Herceptin appear to be related to the amount of HER2 expression on the invasive tumor cell component in the breast cancer. Breast cancer specimens can be formalin fixed, paraffin embedded, frozen or fresh or tissue extracts. On a practical basis clinical labs use paraffin embedded formalin fixed slides. Either the primary tumor or metastatic site may be the source for tumor cells. Usually there is HER2 status concordance of the primary tumor and its metastasis. Simon reported a 95% concordance of a primary tumor and its axillary lymph node mets [27]. However Thor reported a nearly 20% discordance of the primary tumor and its distant visceral mets [28]. If a tumor is HER2 negative, 95% of its mets will be entirely negative for HER2.

While HER2 protein overexpression is seen in about 25% of invasive human breast cancer, the highest overexpression frequency is noted in inflammatory breast cancer (50%) and in up to 70% of DCIS particularly comedo and high grade DCIS subtypes [29]. In mixed tumors with an extensive intraductal component (EIC), the invasive tumor cell component not the DCIS is evaluated for HER2 expression for decisions on therapy.

Serum HER2 Measurements

HER2 extracellular domain (ECD) protein shed into the blood can be detected by an enzyme linked immunosorption assay (ELISA). The test is not applicable in routine clinical labs. The levels of shed ECD do not correlate with tumor burden but may reflect clinical treatment responses. This method lacks validation for general clinical use [5,30].

Clinical Methods for Detection of HER2 Status

A wide variety of assay methods are available to measure gene amplification or protein overexpression. The target molecules for measurement include DNA, mRNA, cell surface protein and shed HER2 ECD. Some assays are not suitable for routine pathology labs. Examples are the Western, Southern, or Northern blotting test and pCR as these require fresh or frozen breast cancer tissue and are expensive, tedious and impractical for routine use [31]. Slamon reported a correlation of results using blotting assays with the results obtained by fluorescence in situ hybridization (FISH) reflecting

gene copy number and immunohistochemistry (IHC) measuring membrane protein levels. Both FISH and IHC are used in routine surgical pathology labs with the tests performed on archival or formalin fixed paraffin embedded specimens or fresh/frozen tissue. Both tests require only small amounts of tumor specimen and non-tumor contents of the specimen do not affect test values. Routine HER2 testing is part of the diagnostic evaluation of invasive breast cancer.

Immunohistochemistry (IHC)

Detection of HER2 Membrane Protein

The most frequently used clinical lab assay to measure HER2 membrane protein is the IHC test. Formalin fixed paraffin embedded tumor slides are used and membrane staining intensity and numbers of stained cells are evaluated. The method is relatively simple and inexpensive. Many variables influence IHC accuracy and reproducibility including age of embedded tissue, tissue fixation technique, sensitivity and specificity of the detection system antibody and subjective individual observer interpretation without a standardized scoring system. Several commercial IHC kits for HER2 IHC testing are available. The FDA approved the HercepTest (Dako Corp) for selecting metastatic breast cancer patients for Herceptin use. The HercepTest uses the A0435 polyclonal antibody and employs a scoring system to standardize interpretation with a score of 1, 2 or 3+ compiled from the percentage of cells stained, pattern of membrane staining (partial or complete) and the staining intensity. A computer assisted image analyzer can be used to add objectivity to the score. The FDA also approved the CB11 monoclonal antibody test (Ventana Medical) for clinical use [32,33]. For research purposes antibody tests capable of detecting the phosphorylated activated receptor in a tumor have been developed but are not for general clinical use.

Fluorescence in situ Hybridization

Detection of HER2 Gene Amplification

FISH detects the HER2 gene copy number in individual tumor cells using paraffin embedded tissue sections. The test uses a fluorescent oligonucleotide probe complimentary to the HER2 gene on chromosome 17 and requires a fluorescence microscope making it more costly and labor intensive than IHC. Old or even poorly fixed paraffin slides can be used as chromosomal DNA is more stable than membrane protein antigen. The FDA has approved two commercial FISH kits – the Ventana Inform® kit and the Vysis Path Vysion® kit. The Vysis test uses the direct labeled probe for the gene and an internal control probe to correct for tumor aneuploidy. The control probe detects the chromosome 17 centromere and a ratio of the HER2 probe number to

centromere number is measured with values greater than 2 positive for amplification. In general, FISH testing is highly sensitive and 100% specific for detecting gene amplification.

Patient Therapy Selection - To FISH or Not

In breast cancer HER2 amplification directly relates to HER2 overexpression of mRNA and protein. Only a small percent of overexpression lacks gene amplification. Paradoxically, however, most reported clinical studies using Herceptin rely on IHC values for HER2 measurement. When IHC is compared to FISH in the same specimen, IHC 3+ levels are concordant with FISH positivity (80-100%) but 2+ cases have concordance only 30% of the time. For IHC scores of 2+, common clinical practice is to test the case by FISH. Retrospective data from multiple clinical trials in metastatic breast cancer show that FISH positivity is the best predictor of clinical benefit from Herceptin and supports the concept of FISH testing to select patients for Herceptin therapy [34,35].

Finally the reproducibility of both FISH and IHC testing in the community depends on the assay volume performed per month by the individual lab. Large volume reference labs have less discrepancy in results. Hence accuracy of lab results is paramount for successful treatment outcomes.

Clinical Studies of Herceptin in Breast Cancer

Phase I and II Clinical Trials

After confirmation of Herceptin's antitumor activity in preclinical studies, Phase I and II clinical trials reconfirmed the safety and efficacy of Herceptin in humans.

Two large trials evaluated the effectiveness of Herceptin as monotherapy in patients with HER2 overexpressing breast cancer. The first trial included 222 patients all heavily treated with prior chemotherapy yet showed a response rate (complete and partial) of 15% with responses lasting 9.1 months [36]. A second trial in patients with less advanced disease reported a response rate (CR + PR) of 26% and clinical benefit in 38% of patients. If stable disease greater than six months was added, the median duration of response was 18.8 months [37]. Hence, Herceptin alone is a reasonable choice for HER2 amplified patients with relatively limited metastatic disease. And when patients progress while on single agent Herceptin, chemotherapy can be added without loss of effectiveness [38].

The Pivotal Trial

In the pivotal trial that led to FDA approval of Herceptin usage in the metastatic setting, Slamon et al compared chemotherapy alone to chemotherapy plus Herceptin in 469 women as first line therapy for advanced metastatic disease [39]. All patients had at least 2+ IHC seen in greater than 10% of their tumor cells. Chemotherapy was an anthracycline (doxorubicin 60 mg/m^2 or epirubicin 75 mg/m^2) plus cyclophosphamide 600 mg/m^2 for those patients who had not received anthracycline in their adjuvant.

For those patients previously treated with adjuvant anthracycline and failed, paclitaxel 175 mg/m^2 was used. All chemo was given every 3 weeks for 6 cycles or longer at investigator discretion and Herceptin given weekly until disease progression. As reflected in Table 1, improvements in time to progression, response rates and one-year survival were seen with the addition of Herceptin to chemotherapy.

Table 1: Chemotherapy (C) vs. Chemotherapy Plus Herceptin (C + H)

	C	C+T	
Response rate	32%	50%	p = 0.001
Time to progression	4.6 mos	7.4 mos	p = 0.0001
Median survival	20.3 mos	25.1 mos	p = 0.046
One-year survival rate	67%	78%	p = 0.01

From Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001;344: 783-792.

The majority of responses were seen in patients with a 3+ IHC value, which was 75% of all patients. Also patients in the chemo only group were eligible to receive Herceptin after disease progression. Two-thirds of this group progressed and crossed over to Herceptin.

These findings of improved RR, TTP and OS are distinctly unusual for advanced breast cancer response and have prompted the early use of Herceptin as 1st line therapy, for HER2 amplified metastatic breast cancer.

Herceptin Chemotherapy Combinations

The response data from the pivotal trial indicated a distinct clinical benefit from adding Herceptin to standard chemotherapy. Preclinical findings from cell line and xenograft models have defined pharmacologic interactions of Herceptin with various chemo agents as being synergistic, additive or antagonistic. The molecular basis underlying

synergistic cytotoxicity is unknown but postulated to be impaired repair of drug induced DNA damage by the Herceptin [26,40]. This data is used as a guide to develop clinical Herceptin/chemotherapy combinations for study. Pegram and colleagues screened a series of chemotherapy agents to define their interaction with Herceptin as shown in Table 2 [41].

Table 2: Interactions of various chemotherapeutic drugs and trastuzumab - preclinical studies

INTERACTION	AGENT	
Synergy	Cisplatin	Carboplatin
	Docetaxel	Vinorelbine
	Etoposide	Thiotepa
Addition	Radiation therapy	Paclitaxel
	Doxorubicin	Vinblastine
	Methotrexate	
Antagonism	5-Fluorouracil	

Oncogene 1999; 18:2241-2251

The taxanes (paclitaxel and docetaxel) have been extensively evaluated in combination with Herceptin. Docetaxel, the most active single agent against breast cancer, exhibits potent synergistic activity with trastuzumab across a range of doses with overall response rates of 45 to 63% and time to progression of nine and twelve months in clinical studies [42,43].

Additionally Slamon and Pegram also showed a triple drug combination of docetaxel, platinum and Herceptin (TCH) demonstrated the most highly synergistic interaction of all regiments tested in preclinical studies [44].

Preliminary results from clinical trials of this 3 drug combination using either Cisplatin (BCIRG 001) or carboplatin (BCIRG 002) revealed response rates of 79% and 56% respectively with a low incidence of grade 3/ 4 toxicity [45].

The most synergistic single chemotherapy drug combined with Herceptin in preclinical studies was vinorelbine (Navelbine). Two Phase II trials have evaluated vinorelbine in HER2 positive metastatic breast cancer. Both drugs were given weekly with overall response rates 78% in the Burstein study and 73% in the Johanzeb trial. Toxicity was mainly neutropenia and an added advantage was lack of drug-induced alopecia [46,47].

Herceptin Dose Schedule

Preclinical pharmacokinetic studies reveal a slow serum clearance and long terminal half-life with a minimum serum trough concentration of 10 μg/ml to achieve therapeutic responses in humans [17,48]. Phase I and II trial data led to the pivotal trial loading dose of 4 mg/kg IV with subsequent weekly doses of 2 mg/kg IV. This is now the most commonly used clinical dose schedule with the loading dose given over 90 minutes and weekly doses over 30 minutes. Studies using higher loading and maintenance doses do not have increased activity but do increase mean half life and decrease Herceptin clearance [49].

Gelmon has reported that a loading dose of 8 mg/kg IV followed every 3 weeks by a maintenance dose of 6 mg/kg give serum concentrations equivalent to standard weekly doses with expected equal effectiveness [50]. This regimen is more convenient than weekly dosing and has been incorporated into standard clinical practice. Subcutaneous administration of Herceptin is under current study to determine pharmacokinetics and safety but is not yet used in clinical practice [51].

Duration of Therapy

In the metastatic setting when Herceptin is used either alone or combined with chemo, usually the cytotoxic chemo agent in continued until best stable response and then stopped and the Herceptin is continued. For some patients the Herceptin will be given for long periods of time, even years. When the cancer does progress, if the patient is still on Herceptin alone, then either a hormonal agent or another chemotherapy drug can be added especially a drug of proven effectiveness like vinorelbine, docetaxel or even a taxane/platin doublet.

For patients who progress on a Herceptin/chemo combination, a different Herceptin/chemo combination is chosen as next line therapy. No good data exists to direct what order to choose drugs for second or third line therapy so clinical judgment is used. For no response to Herceptin either alone or in combination, then the Herceptin is stopped [52,53].

Herceptin Toxicity

Generally Herceptin is a well tolerated drug. Mild reported side effects include a hypersensitivity infusion reaction of fever and chills usually confined to the first infusion and controlled by antihistamines and acetaminophen. Mild airway congestion and diarrhea are also noted. Life threatening acute respiratory failure can occur (even

delayed by 24 hours) in patients with extensive pulmonary impairment. Hematologic toxicity is mild and infrequent.

The side effect of greatest clinical concern is cardiac toxicity. In Phase II trials a 4% risk of cardiac dysfunction was seen in patients treated with Herceptin alone. The highest risk of cardiac dysfunction was seen in the pivotal trial in 27% of patients given Herceptin plus anthracycline and cyclophosphamide [39]. Dysfunction occurred in 13% of patients treated with Herceptin and paclitaxel, in 8% receiving anthracycline plus cyclophosphamide alone and in 1% of the paclitaxel alone group. This dysfunction was manifested by a drop of systolic ejection fraction along with signs and symptoms of CHF including gallop, resting tachycardia, palpitations, weight gain and dyspnea.

The pathogenesis of Herceptin associated cardiotoxicity is not fully understood. HER2 plays a role in embryonic cardiogenesis.

In erbB2 null allele mice, mutant embryos die before day 11 due to dysfunction associated with a lack of cardiac trabeculae and failure of myocyte proliferation [54].

In normal cardiac tissue, HER2 is expressed. But evaluation of HER2 status in heart biopsy tissue from patients with decreased LVF does not show overexpression or amplification. So the Herceptin toxicity is not a direct receptor blockade effect [55]. The Herceptin cardiac toxicity may be predicted by myocardial uptake of radiolabeled trastuzumab as only patients with evidence of uptake developed cardiotoxicity in one series [56].

Chen reported a ventricle restricted erbB2 knockout mouse model that developed dilated cardiomyopathy and impaired response to B-agonists and stress [57]. The mice are rescued by overexpression of BcL-xL and salvage mechanism of apoptosis suggesting that erbB2 plays a role in myocyte survival pathways and erbB2 impairment creates a susceptibility for heart failure in response to a cardiotoxic insult. Based on this data, one theory is that Herceptin amplifies an existing cardiotoxic effect. This may explain why patients previously treated with anthracycline may be susceptible to CHF induced by Herceptin. While anthracycline cardiotoxicity is a dose related side effect, Herceptin associated cardiotoxicity is not dose dependent. Herceptin cardiotoxicity is generally not severe, can be treated successfully and even reversed with discontinuation of drug or by treatment with standard CHF meds even while Herceptin is continued. Anthracyclines cause damage to the myocyte (vacuolization, drop out or frank necrosis) while Herceptin lacks a morphological change on cardiac biopsy [58].

Risk factors for development of Herceptin cardiotoxicity are age > 60 yrs and concurrent administration of Herceptin and Anthracycline. Some believe that any prior anthracycline treatment, left-sided chest wall radiation, and pre-existing cardiac dysfunction are also risk factors.

Warning signs of dysfunction include unexplained tachycardia, weight gain of ≥ 2 kg in one week, and new onset DOE. Cardiac function is assessed by multigated acquisition scanning (MUGA) for LVEF. Echocardiogram is also used to measure systolic and diastolic dysfunction. Guidelines have been published for monitoring cardiac function and to treat heart failure. Ace inhibitors, Beta blockers, digoxin and diuretics are all used and most patients improve and can stay on Herceptin [59].

Table 3

Proposed Guidelines for the Management of Patients Treated with Trastuzumab, Based on Physical Status and LVEF

Physical status[a]	LVEF	Action		
		Trastuzumab	LVEF monitoring	Management
Asymptomatic	↓ but normal	Continue	Repeat in 4 weeks	
	↓ > 10 points but normal	Continue	Repeat in 4 weeks	Consider β-blockers
	↓ 10–20 points and LVEF > 40%	Continue	Repeat in 2 to 4 weeks • Improved: monitor • Not improved: stop trastuzumab	Treat for CHF
	↓ > 20 points to < 40% or LVEF < 30%	Hold	Repeat in 2 weeks • Improved to > 45%: restart trastuzumab • Not improved: stop trastuzumab	Treat for CHF
Symptomatic	↓ < 10 points	Continue		Search for noncardiac pathology (e.g., anemia)
	↓ > 10 points and LVEF > 50%	Continue	Repeat in 2–4 weeks • Stable or improved: continue trastuzumab • Worsened: stop trastuzumab	Treat for CHF
	↓ < 30 points	Stop		Treat for CHF

LVEF: left ventricular ejection fraction; CHF: congestive heart failure.

[a] Heart rate and body weight should be monitored weekly. Asymptomatic is defined as changes in heart rate and or weight (as per Table 3) but without symptoms of dyspnea on exertion. Symptomatic is defined as a new, spontaneous (i.e., unsolicited) report of symptoms of dyspnea on exertion, pulmonary vascular congestion, or edema.

Cancer 1598, 2002:95;7

HER2 and Hormonal Status

Clinically an inverse relationship between ER/PR expression and HER2 overexpression has been noted. ER/PR positive tumors tend to lack HER2 overexpression and conversely, HER2 overexpressing tumors tend to be ER/PR negative. Trial data suggests that breast cancer patients who are ER/PR positive and HER2 positive may have an impaired response to tamoxifen. That is tamoxifen therapy is less effective than would be expected for HER2 overexpressors.

It is now recognized that there are complex interactions between HER2 and the ER. That is, a cross talk exists between HER2 and the ER signal transduction pathways. HER2 overexpression causes signaling with increased phosphorylation of tyrosine residues in the ER with ligand independent ER activation and loss of the inhibitory effect of tamoxifen.

The logical deduction is to block both the ER and HER2 pathways, which has been done in cell lines with results of greater growth inhibition that single pathway blockade [60].

This concept is further supported by a recent report that ER+, HER1+ and/or HER2+ primary breast cancer responded better to letrozole (aromatase inhibitor) than to tamoxifen [61].

Herceptin Resistance

Even in patients with high levels of HER2 overexpression, the Herceptin response rate is limited and in responders, resistance eventually occurs. The mechanisms of resistance are poorly understood and are likely complex since there are multiple explanations as to the antitumor activity of the antibody. Alterations of the HER2 receptor, other mutation changes in the downstream signaling pathway, decreased immune function of patients limiting ADCC are all possible. Also likely is the fact that for many breast cancers, the HER2 activation pathway in and of itself is not solely responsible for the malignant growth, proliferation and survival of the cell. So targeting one receptor will not be enough to achieve sustained clinical responses. It may be important to target other members of the EGFR family simultaneously with HER2. For instance, dual blockade of HER1 (EGFR) and HER2 results in improved growth inhibition in breast cell lines [62]. Additionally, there is evidence that the IGF-1R (insulin like growth factor receptor 1) signaling inhibits the Herceptin response in HER2 overexpressing cells due to opposing effects on p27 levels. So treatments that decrease IGF-1R signaling may prevent resistance to Herceptin [63].

Future Directions

There are many questions to be answered in the use of Herceptin:

1. The role of Herceptin in adjuvant and neoadjuvant chemotherapy
2. Which hormonal agents and in what sequence to combine with Herceptin?
3. Which chemotherapy drugs and what sequences are the most effective in neoadjuvant, adjuvant and the metastatic setting?
4. What is the optimal duration of therapy?
5. How can cardiotoxicity be minimized and what will be the long term effects on the heart?
6. What combination with other blockers of signal transduction are best to achieve maximal tumor control?
7. How can we profile the patients best suited to different combinations of receptor blockade?

What is clear for now is that the development of Herceptin and its rapid introduction into the clinical arena has opened the door in the search for biologically based and individualized therapies in breast cancer.

REFERENCES

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA Cancer J Clin. 2003;53:5-26

2. Polychemotherapy for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet. 1998;352:930-942

3. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235:177-182

4. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001;2:127-137

5. Nunes RA, Harris LN. The HER2 extracellular domain as a prognostic and predictive factor in breast cancer. Clin Breast Cancer. 2002;3:125-135; discussion 136-127

6. Burden S, Yarden Y. Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. Neuron. 1997;18:847-855

7. Oved S, Yarden Y. Signal transduction: molecular ticket to enter cells. Nature. 2002;416:133-136

8. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, Yarden Y. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. Genes Dev. 1998;12:3663-3674

9. Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell. 1988;54:105-115

10. Di Fiore PP, Pierce JH, Kraus MH, Segatto O, King CR, Aaronson SA. erbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells. Science. 1987;237:178-182

11. Ignatoski KM, Maehama T, Markwart SM, Dixon JE, Livant DL, Ethier SP. ERBB-2 overexpression confers PI 3' kinase-dependent invasion capacity on human mammary epithelial cells. Br J Cancer. 2000;82:666-674

12. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc Natl Acad Sci U S A. 1992;89:10578-10582

13. Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Stem Cells. 1998;16:413-428

14. Press MF, Bernstein L, Thomas PA, Meisner LF, Zhou JY, Ma Y, Hung G, Robinson RA, Harris C, El-Naggar A, Slamon DJ, Phillips RN, Ross JS, Wolman SR, Flom KJ. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol. 1997;15:2894-2904

15. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989;244:707-712

16. Pietras RJ, Pegram MD, Finn RS, Maneval DA, Slamon DJ. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. Oncogene. 1998;17:2235-2249

17. Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME, Shepard HM. Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc Natl Acad Sci U S A. 1992;89:4285-4289

18. Drebin JA, Link VC, Stern DF, Weinberg RA, Greene MI. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. Cell. 1985;41:697-706

19. Sarup JC, Johnson RM, King KL, Fendly BM, Lipari MT, Napier MA, Ullrich A, Shepard HM. Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth. Growth Regul. 1991;1:72-82

20. De Santes K, Slamon D, Anderson SK, Shepard M, Fendly B, Maneval D, Press O. Radiolabeled antibody targeting of the HER-2/neu oncoprotein. Cancer Res. 1992;52:1916-1923

21. Reese D, Arbolede J, Twaddell J, al. e. Effects of 4D5 antibody on HER2/neu heterodimerization with other class I receptors in human breast cancer cells. Proc Am Soc Clin Oncol. 1996;37 (Abstract 353)

22. Lane HA, Motoyama AB, Beuvink I, Hynes NE. Modulation of p27/Cdk2 complex formation through 4D5-mediated inhibition of HER2 receptor signaling. Ann Oncol. 2001;12 Suppl 1:S21-22

23. Segatto O, King CR, Pierce JH, Di Fiore PP, Aaronson SA. Different structural alterations upregulate in vitro tyrosine kinase activity and transforming potency of the erbB-2 gene. Mol Cell Biol. 1988;8:5570-5574

24. Hotaling TE, Rutz B, Wolfgang-Kemball D, al. e. The humanized anti HER2 antibody rhuMab HER2 mediates antibody dependent cell mediated cytotoxicity via FcgR111. proc Am Assoc Cancer Res. 1996;37:471 (Abstract)

25. Pegram M, Baly D, Wirth C, al. e. Antibody dependent cell mediated cytotoxicity in breast cancer patients in phase III clinical trials of a humanized anti-HER2 antibody. Proc Am Assoc Cancer Res. 1997;38:602 (Abstr)

26. Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB, Slamon DJ. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene. 1994;9:1829-1838

27. Simon R, Nocito A, Hubscher T, Bucher C, Torhorst J, Schraml P, Bubendorf L, Mihatsch MM, Moch H, Wilber K, Schotzau A, Kononen J, Sauter G. Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. J Natl Cancer Inst. 2001;93:1141-1146

28. Edgerton SE, Merkel D, Moore DH, Thor AD. HER-2/new/erb B-2 status by immunohistochemistry and FISH: clonality and progression with recurrence and metastasis. Breast Cancer Res Treat. 2000;64:55 (Abstract)

29. Moreno A, Lloveras B, Figueras A, Escobedo A, Ramon JM, Sierra A, Fabra A. Ductal carcinoma in situ of the breast: correlation between histologic classifications and biologic markers. Mod Pathol. 1997;10:1088-1092

30. Hayes D. Is There a Role for Monitoring Circulating HER-2? Clin Breast Cancer. 2002;3:136-137

31. Van de Vijuer MJ. Techniques for the Detection of HER2 Gene Amplifications and Overexpression. HER2 in Oncology. 2000;1:2-4

32. Schaller G, Evers K, Papadopoulos S, Ebert A, Buhler H. Current use of HER2 tests. Ann Oncol. 2001;12 Suppl 1:S97-100

33. Vang R, Cooley LD, Harrison WR, Reese T, Abrams J. Immunohistochemical determination of HER-2/neu expression in invasive breast carcinoma. Am J Clin Pathol. 2000;113:669-674

34. Horton J. Trastuzumab use in breast cancer: clinical issues. Cancer Control. 2002;9:499-507

35. Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L. Evaluation of HER-2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J Clin Oncol. 2002;20:3095-3105

36. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ. 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. 1999;17:2639-2648

37. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol. 2002;20:719-726

38. Burris HA, Hansworth JD, Miranda FT. Phase II trial of Herceptin induction followed by combination therapy with paclitaxel and Carboplatin; a Minnie Pearl Research Network Trial. Breast Cancer Res Treat. 2000;64

39. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344:783-792

40. Arteaga CL, Winnier AR, Poirier MC, Lopez-Larraza DM, Shawver LK, Hurd SD, Stewart SJ. p185c-erbB-2 signal enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: association between an oncogenic receptor tyrosine kinase and drug-induced DNA repair. Cancer Res. 1994;54:3758-3765

41. Pegram M, Hsu S, Lewis G, Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinavar F, Slamon D. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene. 1999;18:2241-2251

42. Esteva FJ, Valero V, Booser D, Guerra LT, Murray JL, Pusztai L, Cristofanilli M, Arun B, Esmaeli B, Fritsche HA, Sneige N, Smith TL, Hortobagyi GN. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. J Clin Oncol. 2002;20:1800-1808

43. Kuzur ME, Albain RS, Huntington MO, al. e. A Phase Ii trial of docetaxel and Herceptin in metastatic breast cancer patients overexpressing HER-2. Proc Am Soc Clin Oncol. 2000;19:131

44. Slamon D, Pegram M. Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials. Semin Oncol. 2001;28:13-19

45. Nabholtz JM, Pienkowski T, Northfelt D, al. e. Results of two open label multi center phase two pilot studies with Herceptin in combination with docetaxel and platinum salts (cis or carboplatin) TCH as therapy for advanced breast cancer (ABC) in women with tumor over-expressing the HER2 neu proto-oncogene. Eur J Cancer. 2001;37:190

46. Burstein HJ, Kuter I, Campos SM, Gelman RS, Tribou L, Parker LM, Manola J, Younger J, Matulonis U, Bunnell CA, Partridge AH, Richardson PG, Clarke K, Shulman LN, Winer EP. Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. J Clin Oncol. 2001;19:2722-2730

47. Jahonzeb M, Mortimer J, Yunus F, al. e. A Phase II multicenter trial of weekly Herceptin with Navelbine in chemonaive patients with HER2 positive metastic breast cancer. Eur J Cancer. 2001;37 (supp. 6):194

48. Tokuda Y, Ohnishi Y, Shimamura K, Iwasawa M, Yoshimura M, Ueyama Y, Tamaoki N, Tajima T, Mitomi T. In vitro and in vivo anti-tumour effects of a humanised monoclonal antibody against c-erbB-2 product. Br J Cancer. 1996;73:1362-1365

49. Washington CB, Lieverman G, P. L, al. e. A population pharmacokinetic model for trastuzumab following weekly dosing. Clin Pharmacol Ther. 2002;71:12

50. Gelmon K, Arnold A, Verma S, al. e. Pharmacokenitics (pk) and Safety of Trastuzumab(Herceptin [R]) when administered every three weeks to women with metastatic breast cancer. Proc Am Soc Clin Oncol. 2001;20:271

51. Leyland-Jones B, Arnold A, Gelmon K, Verma S, Ayoub JP, Seidman A, Dias R, Howell J, Rakhit A. Pharmacologic insights into the future of trastuzumab. Ann Oncol. 2001;12 Suppl 1:S43-47

52. Mackey J, Gelmon K, Verma S, al. e. Continued use of Herceptin after disease progression in women with HER-2 positive (HER2+) metastatic breast cancer (MBC): results from a retrospective analysis of 105 cases. Proc Am Soc Clin Oncol. 2002;Abst 207

53. Gelmon K. Response to first Herceptin containing regimen does not predict response to second Herceptin containing regimen: A rationale for Herceptin Beyond Disease Progression. Peer View Press 2002. 2002

54. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature. 1995;378:394-398

55. Fuchs I, Landt S, Bretcer H, al. e. HER2 expression in the myocarduim as a cause for cardiotoxicity of trastuzumals, (Herceptin). Proc Am Soc Clin Oncol. 2000;19:102a

56. Behr TM, Behe M, Wormann B. Trastuzumab and breast cancer. N Engl J Med. 2001;345:995-996

57. Chien KR. Myocyte survival pathways and cardiomyopathy: implications for trastuzumab cardiotoxicity. Semin Oncol. 2000;27:9-14; discussion 92-100

58. Ewer S, Vooletich M, Valero V, al. e. Trastuzumab (Herceptin) Cardiotoxicity: Clinical Course and Cardiac Biopsy Correlations. Proc Am Soc Clin Oncol 2002. 2002

59. Keefe DL. Trastuzumab-associated cardiotoxicity. Cancer. 2002;95:1592-1600

60. Witters LM, Kumar R, Chinchilli VM, Lipton A. Enhanced anti-proliferative activity of the combination of tamoxifen plus HER-2-neu antibody. Breast Cancer Res Treat. 1997;42:1-5

61. Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E, Borgs M. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol. 2001;19:3808-3816

62. Normanno N, Campiglio M, De LA, Somenzi G, Maiello M, Ciardiello F, Gianni L, Salomon DS, Menard S. Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. Ann Oncol. 2002;13:65-72

63. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst. 2001;93:1852-1857