

I. THE IMPORTANCE OF HER2 IN BREAST CANCER

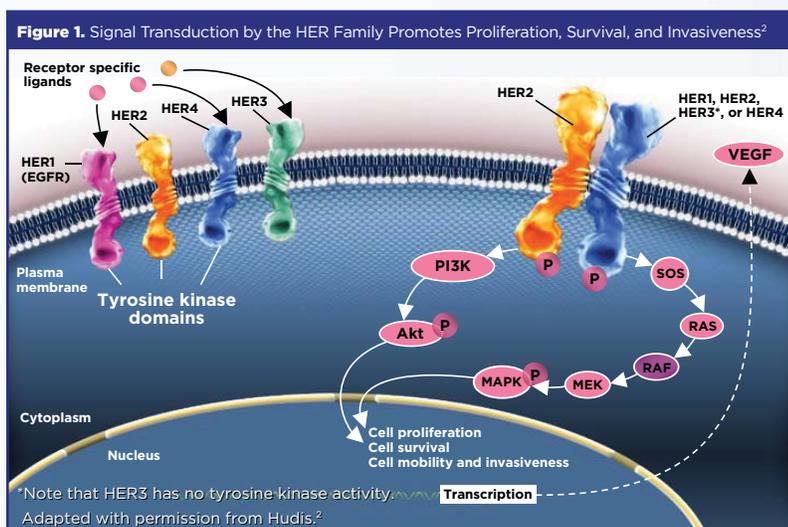
The human epidermal growth factor receptors (HER), also known as ERBB receptors, are a family of signal transduction proteins. There are 4 family members in humans—HER1, HER2, HER3, and HER4—each of which is composed of an extracellular (ligand-binding) domain, a transmembrane domain, and an intracellular tyrosine kinase, except for HER3, which lacks a tyrosine kinase domain (Figure 1).^{1,2} HER-family receptors become active upon interaction with ligand and subsequent dimerization, followed by activation of downstream signaling proteins by phosphorylation. In humans, the 4 HER proteins interact with a range of ligands, although HER2 has no known activating ligands.² For example, HER1 interacts with at least 6 ligands: epidermal growth factor (EGF), transforming growth factor α (TGF α), amphiregulin, heparin-binding EGF-like growth factor, betacellulin, and epiregulin.³ HER2, also referred to as ERBB2, NEU, or HER2/*neu*, can act as a dimerization partner with the other HER receptor proteins. As there are no known activating ligands for HER2, its activity is a consequence of its dimerization with other HER-family receptors as well as its homodimerization.³ Upon activation, the tyrosine kinase domain of each of the HER-family receptors can activate downstream signaling molecules, such as those in the PI3K/Akt and RAS/RAF/MEK/MAPK pathways.²

HER2 is encoded by the *HER2* gene, which is located on chromosome 17. Because of its function as an activator of signaling pathways, HER2 plays a central role in a number of cellular processes, including proliferation, motility, and resistance to apoptosis. As mentioned above, HER2 has no known ligand and can heterodimerize with other HER proteins, thus allowing HER2 to participate in a number of signal transduction pathways in the absence of a specific ligand.³ This effect may be enhanced by the overexpression of HER2 in cancer cells, leading to increased cell proliferation and decreased cell death, as well as changes in cell motility.

The association of HER2 protein overexpression or *HER2* gene amplification with cancer, notably with breast cancer, was reported over 20 years ago.⁴ Approximately 10% to 34% of breast cancers overexpress the HER2 receptor and are referred to as HER2-positive (HER2+).¹ While it has not yet been definitively proven, it appears likely that overexpression of HER2 protein is linked with *HER2* gene amplification, and the incidence of overexpression in the presence of a single copy of the *HER2* gene is rare.⁵ Expression levels of HER2 are significantly different between tumor cells with an amplified *HER2* gene and those without amplification, with the former ranging from 500,000 to 2,000,000 receptors per tumor cell, and the latter ranging from 25,000 to 185,000 receptors per tumor cell.⁵ As will be discussed below, HER2 status provides both prognostic and predictive information in patients with breast cancer, and HER2 forms the basis for targeted agents that have transformed the treatment of breast cancers with amplified *HER2* or overexpressed HER2.

HER2 STATUS AND PROGNOSIS IN BREAST CANCER

Overexpression of the HER2 receptor is associated with poor prognosis in patients with breast cancer, as well as with aggressive tumor growth and metastases.³ HER2 positivity has also been associated with tumor grade,^{1,6} positive lymph node metastases at presentation,¹ and mitotic count.⁶ HER2+ tumors are



less likely to be hormone receptor (estrogen receptor [ER] or progesterone receptor [PgR])-positive.¹

HER2 status also correlates with relative response to various agents. HER2 positivity may result in increased resistance to endocrine therapy and with a decreased benefit from non-anthracycline, non-taxane-containing chemotherapy.⁷ Conversely, HER2-positive patients may exhibit improved response to anthracycline therapy, as well as to paclitaxel.⁷

Data surrounding the prognostic and predictive value of HER2 status are continually evolving. In a recent study, it was shown that higher levels of *HER2* gene amplification were associated with worse outcomes in patients treated with doxorubicin-based therapy in the adjuvant setting.⁸

The association of *HER2* gene amplification or HER2 overexpression with some breast cancers has allowed for the development of agents that specifically target HER2, altering the treatment landscape for these cancers. Trastuzumab, which was approved for the treatment of metastatic breast cancer in 1998, for the adjuvant treatment of lymph node-positive breast cancer in 2006, and for the adjuvant treatment of lymph node-negative breast cancer in 2008, is a humanized monoclonal antibody to the HER2 protein. Lapatinib is a selective inhibitor of the tyrosine kinase activity of HER2 and EGFR. Each of these agents has shown efficacy in patients whose tumors are HER2+.

HER2 EXPRESSION AND BREAST PATHOLOGY

An association of HER2 status with various breast pathologies has been noted. For ductal carcinoma in situ (DCIS), the incidence of HER2+ status is higher than that seen for invasive breast cancer (approximately 24%, vs 38% for DCIS). HER2 overexpression in this pathologic subtype is associated with higher grade and more extensive forms of DCIS.¹ *HER2* gene amplification is seen less often—in invasive lobular carcinoma in approximately 10% of cases—but is linked to adverse outcomes. HER2 positivity is present less frequently in male breast cancer than in female breast cancer. The rate of HER2 positivity is also

very low to nonexistent in mucinous, medullary, and tubular carcinomas; breast sarcomas and Phyllodes tumors; as well as in hereditary breast cancer, which is associated with mutations in *BRCA1/BRCA2*. A low level of HER2 expression has been observed in some benign breast disease biopsies and is associated with a greater risk for subsequent development of invasive breast cancer.¹

HER2 STATUS IN PRIMARY VS METASTATIC BREAST CANCER

Recent studies have shown some discordance between HER2 status of the primary tumor and metastatic lesions in the same patient. Discordance rates are 20% to 30%¹ and may be at least partly due to issues of reproducibility of HER2 assays, discussed below. However, HER2 status may evolve, and HER2 positivity or negativity may develop between the time of primary and metastatic disease. The question of the stability of HER2 status is currently uncertain, but data continue to emerge to address this issue. A recent study showed a concordance rate of 66% in HER2 status between the primary and metastatic tumors.⁹

II. HER2 TESTING

As mentioned above, tumor HER2 status can provide both prognostic and predictive information in patients with breast cancer. Knowledge of HER2 status is also essential in the decision of whether to treat with one of the HER2-targeted agents, trastuzumab or lapatinib. For this reason, it has been recommended that HER2 testing be performed for all newly diagnosed invasive breast cancers. Currently, the methods used most often for HER2 testing are immunohistochemistry (IHC) and in situ hybridization, described below. These assays are most often performed on formalin-fixed, paraffin-embedded (FFPE) tissue samples.

SLIDE-BASED ASSAYS

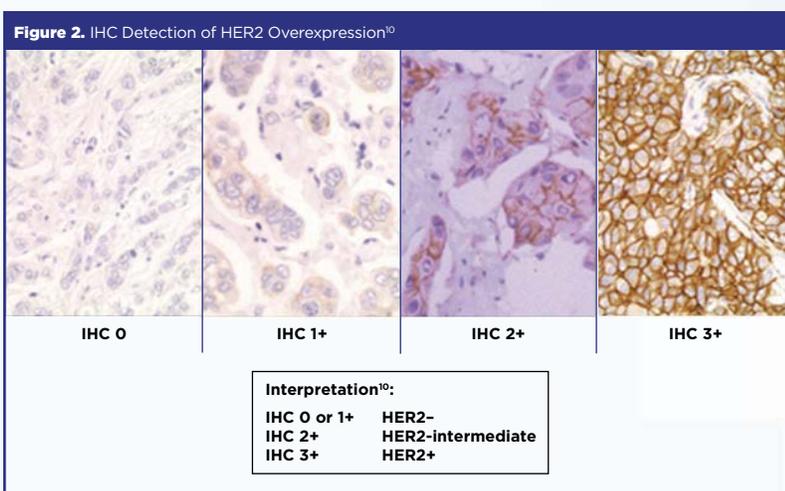
Immunohistochemistry

Immunohistochemical staining allows for the detection of protein in tissues and is the most frequently performed initial test of HER2 status in patients with

newly diagnosed invasive breast cancer. Currently, there are 2 commercially available US Food and Drug Administration (FDA)-approved HER2 IHC assays: Dako HercepTest™ (Dako Corporation, Glostrup, Denmark) and Ventana Pathway™ (Ventana Medical Systems, Tucson, AZ). Because the HER2 protein is expressed in normal breast epithelial cells, the HER2 IHC assay is a quantitative, rather than a qualitative, test. One of the most widely used guidelines for interpretation of IHC results is that developed by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).⁷ For IHC, a positive HER2 test is defined as 3+ cell surface protein expression (uniform intense staining of >30% of invasive tumor cells), an equivocal test as 2+ cell surface protein expression (complete membrane staining that is variable or weak intensity, but with cell surface-associated staining in >10% of cells), and a negative test as 1+ (weak or incomplete staining in any proportion of tumor cells) or 0 (no staining) (Figure 2¹⁰).

While IHC is the most commonly used initial assay for HER2, this method suffers from a number of disadvantages. Many of these disadvantages are due to technical issues, such as variations in tissue fixation, tissue processing, and embedding of tissue in paraffin.¹ One crucial issue is the difficulty in standardization of signal, which can be at least partly due to variable fixation and to variation in antigen retrieval.⁵ Variability may also be introduced depending on the type of antibody (monoclonal vs polyclonal) being used. Another key issue for IHC is that of interpretation, as this is somewhat subjective and will differ depending on the slide scorer.

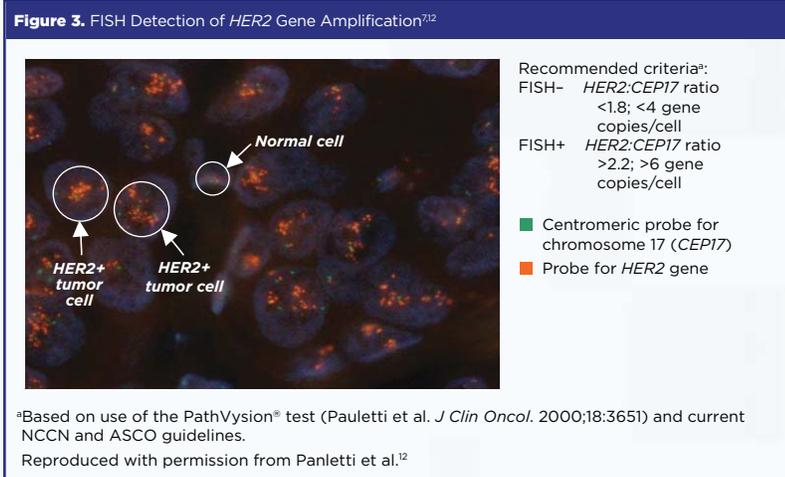
Some of these issues can be addressed; for example a recent study showed that fixation issues can be ameliorated.¹¹ This study, in which a standard fixation time of 6 hours was utilized, showed that there was a decrease in inconclusive cases (from 10.8% to 3.4%). While improvements can be made, there is also the option of using the alternative technique of fluorescence in situ hybridization (FISH), described in the next column.



Fluorescence In Situ Hybridization

The overexpression of the HER2 protein is often the result of gene amplification. IHC can directly measure protein expression, while FISH can assess gene amplification. This method utilizes fluorescent probes to detect the *HER2* gene and can also utilize a chromosome 17 probe (*CEP17*), which acts as an internal control. There are 3 FDA-approved FISH assays to assess *HER2* amplification status: PathVysion™ (Abbott Laboratories, Abbott Park, IL), INFORM (Ventana Medical Systems, Tucson AZ), and PHarmDX (Dako, Glostrup, Denmark). Two of these assays utilize the *CEP17* probe, while the third (INFORM) assesses only the *HER2* copy number (Figure 3^{7,12}).⁵

As with IHC, scoring of this assay may vary with the guidelines used by the laboratory. The ASCO/CAP



scoring guidelines are as follows⁷: Results are reported as positive for HER2 (average of >6 copies of the *HER2* gene per nucleus, or *HER2/CEP17* ratio of >2.2), equivocal for HER2 (average of 4.0 to 6.0 copies of the *HER2* gene per nucleus, or *HER2/CEP17* ratio of 1.8 to 2.2), and negative for HER2 (average of <4.0 copies of the *HER2* gene per nucleus, or *HER2/CEP17* ratio of <1.8).

Testing and interpretation may be complicated by the presence of polysomy 17, which can confound results of the FISH assay.⁶ The impact of polysomy 17 on response to trastuzumab is still unclear,⁶ and the determination of chromosome 17 copy number should be assessed for patients with breast cancer in order to make the most informed decisions.¹³

While FISH is considered the gold standard of *HER2* assessment, there are crucial disadvantages associated with this method, including increased cost relative to IHC, longer time required for slide scoring, the need for a fluorescent microscope, and the instability of the fluorescent signal, which precludes storing slides for later review.¹

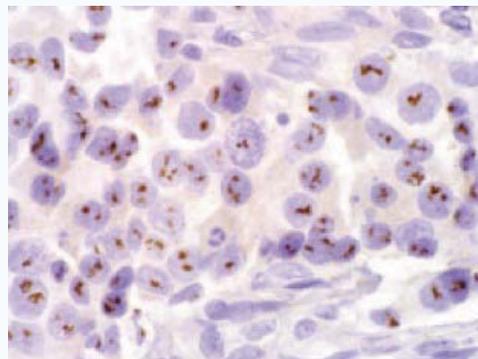
IHC vs FISH

IHC and FISH each offer advantages. The key advantage of IHC is the lower cost, while the key advantage of FISH is the higher objectivity and reproducibility. Fixation appears to be less of an issue with FISH.⁵ A review of relevant literature showed a discordance rate between IHC and FISH of 2% to 20%.⁵ However, FISH has been shown to be superior to IHC in predicting response to the HER2-targeted agent trastuzumab.⁵ As seen below, if an equivocal result is obtained with IHC, an appropriate follow-up is to carry out a FISH assay.

CISH and SISH

As discussed above, FISH offers the advantages of having a chromosome 17 internal control, and being relatively objective. However, FISH suffers from the key disadvantages of requiring a fluorescent microscope and utilizing a signal that fades over time, which precludes the storage and later reassessment of slides. Recently, methodologies that are similar to, but offer

Figure 4. CISH Detection of *HER2* Amplification¹⁶



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- Offers the advantage over FISH of chromogenic detection—can use light microscope¹
- High concordance between FISH and CISH¹⁶
 - Concordance of 95% in a study of 69 IHC 0/1+, 135 IHC 2+, and 50 IHC 3+ cases

advantages over, FISH have become available for the detection of the *HER2* gene in tissue samples. These methods include chromogenic in situ hybridization (CISH) and silver in situ hybridization (SISH). Both CISH and SISH rely on methods similar to those of FISH, but avoid some of the drawbacks of FISH. For either of these methods, a bright-field microscope is used to detect signal, and the signal is stable over time. Studies have shown a high level of agreement between FISH and CISH assay results. In one study, the agreement between FISH and CISH, in terms of whether the *HER2* gene was amplified or not amplified, was 98.6%.¹⁴ Another study showed that correlation between CISH and SISH and correlation of each with patient outcome are high, with concordance rates of approximately 95%.¹⁵ Both CISH and SISH in this study were similar to IHC in predicting cancer-specific survival (Figure 4^{1,16}).¹⁵

Central vs Local Laboratory Testing

Studies have shown that HER2 tests performed at the pathology department of the primary treatment site may often be incorrect, with some studies showing approximately 20% disagreement between local laboratory results and those of a central laboratory.^{17,18} In general, accurate HER2 results are more likely to be provided by laboratories that perform a high volume of tests.

The 2007 ASCO/CAP Guidelines and Updates

ASCO and CAP developed a set of guideline recommendations for optimal HER2 testing performance.⁷ These recommendations addressed

issues of patient selection for testing; a testing algorithm; procedures to reduce assay variation; definition of positive, negative, or equivocal values for both IHC and FISH; and standards for laboratories carrying out testing. One recommendation was for all patients with invasive breast cancer to be tested for the HER2 status of their tumor, utilizing IHC or FISH, following the testing algorithms shown in Figures 5 and 6.⁷ Key to these algorithms is that equivocal results, such as a *HER2/CEP17* ratio of between 1.8 and 2.2, require additional action, as shown. This differs somewhat from the FDA recommendation; if a *HER2:CEP17* ratio between 1.8 and 2.2 is obtained, a minimum of 20 additional cells should be scored by the same scorer, and a second scorer should count a minimum of 40 cells.⁵ If the ratios from these scorers are not in agreement, the entire assay should be repeated. It was recommended by the ASCO/CAP committee that testing be done in labs that are CAP-accredited, or that meet criteria for accreditation and testing requirements.

The most recent CAP updates address the issue of genetic heterogeneity (GH) in HER2 testing.¹⁹ GH exists in a significant number of breast cancer tumors and may confound interpretation. It was recommended that GH be defined as >5% but <50% of infiltrating tumor cells being HER2+ by FISH analysis, and that clusters of cells with amplification of the *HER2* gene should be specifically reported. It is not yet known what the clinical significance of GH is in terms of the benefit of HER2-targeted agents.

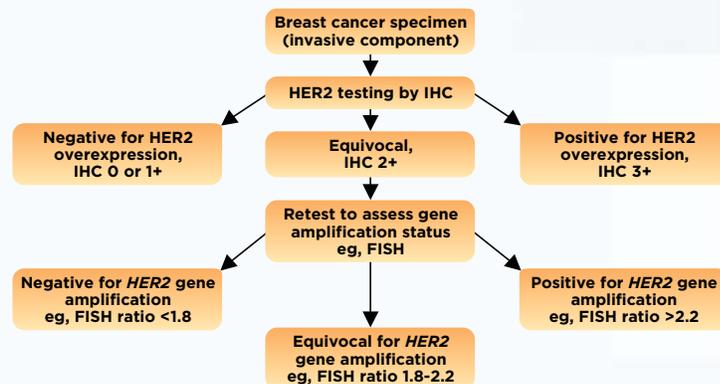
NON-SLIDE BASED ASSAYS

IHC and in situ hybridization assays each utilize tumor tissue that has been fixed and paraffin-embedded. These are currently the primary methods for assessing HER2 status for determining eligibility for HER2-targeted agents. Other methods are in development, as described below.

mRNA by RT-PCR

Reverse transcription polymerase chain reaction (RT-PCR) can be used to assess the relative levels of *HER2* mRNA. Although large scale studies have

Figure 5. HER2 Testing in Clinical Practice: 2007 ASCO/CAP Algorithm—IHC⁷



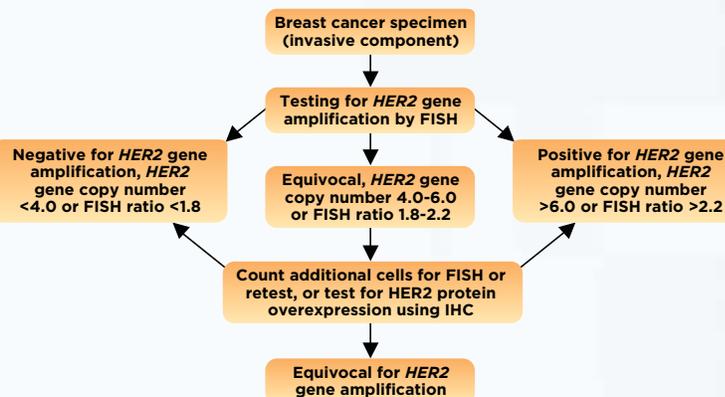
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not yet been performed utilizing this technique to assess HER2 status, it has significant potential, as it is relatively low-cost and rapid.¹ Oncotype DX™, discussed below, utilizes this technique.

mRNA by Microarray

Microarray-based mRNA measurements can be used to simultaneously assess relative levels of a large number of different mRNA molecules. Various multigene predictor assays exist for use in breast cancer management, including Oncotype DX™, MammaPrint™, and TargetPrint™. These assays utilize levels of *HER2* mRNA, as well as levels of mRNA associated with other genes related to HER2 expression, in determining breast cancer risk or recurrence risk. This assay method also shows promise as a rapid, reliable way to determine HER2 status in patients with breast cancer.¹

Figure 6. HER2 Testing in Clinical Practice: 2007 ASCO/CAP Algorithm—FISH⁷



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Dimerization Assays

Dimerization assays directly measure the level of HER2 homodimers. This method has been commercialized in the HERMark™ assay. Results seen thus far show great promise for this approach in predicting response to trastuzumab. Data presented at recent congresses have shown a significant correlation of high levels of HER2 protein (H2T) and HER2 homodimers (H2D) with longer time to progression (TTP) after treatment with trastuzumab.^{20,21}

Phosphorylated HER2

The association of HER2 phosphorylation status (activated or phosphorylated receptor vs unactivated or nonphosphorylated receptor) with prognosis or response to therapy for patients with breast cancer is not yet well-established, although studies have been carried out to address this issue. One large study showed that phosphorylated HER2 was correlated with a higher number of positive lymph nodes, cellular proliferation, and poor prognosis.²² Another study showed that presence of phosphorylated HER2 was associated with resistance to taxane therapy for patients with metastatic breast cancer.²³ Monoclonal antibodies have been developed to detect phosphorylated HER2 and could be used in a standard IHC assay to specifically detect activated HER2 protein.

Tissue and Serum Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA can be used to quantitate the concentration of the extracellular domain (ECD) of the HER2 protein. The ECD is cleaved from the surface of cells by matrix metalloproteases and released into the serum.²⁴ The assessment of ECD in serum avoids many of the problems associated with IHC and in situ hybridization, such as those of fixation, embedding, and storage. One HER2 ELISA is commercially available, the Oncogene Science HER2/neu ELISA (Oncogene Science, Cambridge, MA). The use of ELISA to determine HER2 ECD levels has been approved by the FDA for the monitoring of disease in patients with HER2+ breast cancer.¹ While some studies have shown a correlation between ECD levels and response to specific therapies, a recent meta-analysis showed that assessment of

HER2 ECD levels may not be informative. A pooled analysis of 4 trials showed that baseline ECD levels were not reliably predictive (positively or negatively) of response to therapy. There was a trend toward lower levels of HER2 ECD in patients who achieved better responses, but this was not significant; and there was little change in levels of HER2 ECD before disease progression.²⁴ The potential for the use of serum ELISA exists in both monitoring breast cancer and in making treatment decisions, but more research needs to be carried out in this area before conclusions about the utility of HER2 ECD assessment can be made.

HER2 Testing of Circulating Tumor Cells

To assess HER2 status by either IHC or FISH, specimens from tumor tissue are used. There have been a number of recent studies to investigate the utility of using circulating tumor cells (CTCs) to predict response to therapy, as well as to assess HER2 status without requiring collection of a tissue specimen. It also has been hypothesized that CTCs may play a role in the process of metastasis,²⁵ so CTCs could potentially be used in treatment management decisions for patients with breast cancer. Counting CTCs as a predictor of response to therapy has been validated, but the use of CTCs to assess HER2 status is less well-studied.¹ One study showed that 8 of 21 patients whose primary tumor was HER2 negative (HER2-) or of unknown HER2 status had detectable CTCs that exhibited *HER2* amplification.²⁶ Another study demonstrated that HER2+ CTCs are associated with poor outcome in patients with stage I to stage III breast cancer, with a significant correlation between the presence and number of HER2+ CTCs and decreased disease-free survival and overall survival.²⁵ The authors of this study state that evaluation of HER2 status in CTCs may provide for “real time” assessment of HER2 status in patients with breast cancer. In a study of 24 patients whose primary tumors were HER2-, 9 patients had CTCs that were HER2+. Four of these patients were treated with trastuzumab-containing therapy; 1 had a complete response, and 2 had a partial response.²⁷ These studies provide intriguing data about the use of CTCs to assess HER2 status in patients with breast cancer, and more research is needed to provide answers concerning the value of assessing CTC HER2 status.

III. HER2-TARGETED AGENTS IN BREAST CANCER

As discussed above, it has been shown the HER2 overexpression is associated with a subset of breast cancers, and that HER2 overexpression (“positivity”) is generally associated with poor relative prognosis. Though the poor relative prognosis is problematic, this association led to the opportunity to develop treatments for breast cancer that would specifically target HER2. The first of these was trastuzumab, a humanized monoclonal antibody to HER2. Initial clinical trials of trastuzumab utilized this antibody in patients with metastatic breast cancer, and showed the clinical activity of this agent alone and then in combination with chemotherapy. More recent trials have been carried out in both the adjuvant and neoadjuvant settings. Trastuzumab is now approved for treatment of HER2+ metastatic breast cancer, and in the adjuvant setting for both lymph node-positive and lymph node-negative breast cancer. Lapatinib is the second HER2 targeted agent to be approved for use in patients with breast cancer. Lapatinib is a reversible inhibitor of both HER2 and EGFR tyrosine kinases and has clinical activity in the treatment of metastatic breast cancer. Its mechanism of action differs from that of trastuzumab, and lapatinib is active in patients with progression on the antibody. Lapatinib was approved by the FDA in 2007 for use in patients with metastatic breast cancer that overexpresses HER2 and has progressed on prior chemotherapy and trastuzumab.

HER2-TARGETED AGENTS IN THE METASTATIC SETTING

Trastuzumab Monotherapy

The humanized anti-HER2 monoclonal antibody, eventually called trastuzumab, was shown to inhibit the growth of breast cancer cells, indicating that it had potential as a treatment for breast cancer. In 1996, a phase 2 study was carried out to assess the efficacy and safety of this agent in the treatment of HER2-overexpressing metastatic breast cancer.²⁸ In this study, 46 patients who had received extensive prior therapy for metastatic breast cancer were treated with

intravenously administered trastuzumab (a loading dose of 250 mg, then 100 mg/wk). An overall response rate of 11.6% (5 of 43 assessable patients) was obtained, with 1 patient achieving a complete response. This study was followed by a larger single-arm trial, in which 222 patients with HER2-overexpressing metastatic breast cancer were enrolled.²⁹ In this study, patients received a loading dose of 4 mg/kg intravenously, followed by 2 mg/kg, administered weekly. Responses were once again noted in a number of patients: 8 (4%) patients achieved a complete response, and 26 (12%) patients achieved a partial response.

The efficacy of single-agent trastuzumab in patients with HER2+ breast cancer was confirmed in subsequent trials, including one in which patients were randomized to 2 different doses of trastuzumab: a 4-mg/kg loading dose followed by 2 mg/kg weekly, or an 8-mg/kg loading dose followed by 4 mg/kg weekly.³⁰ Efficacy was similar between the treatment arms. However, the results from this trial established the value of FISH as a method for selecting patients for therapy with trastuzumab: Responses were seen in 34% of patients who were HER2+ by FISH, and 7% in those who were HER2- by FISH.³⁰ A subsequent study was designed to assess whether trastuzumab would be effective and safe if administered every 3 weeks, instead of every week.³¹ Although this was not a randomized trial, the response rate in this study—19 of 83 (23%) patients—was similar to those of prior studies of trastuzumab monotherapy. These trials validated the efficacy of trastuzumab monotherapy and suggested that a range of doses and either weekly or 3-weekly dosing were appropriate.

Chemotherapy in Combination With Trastuzumab

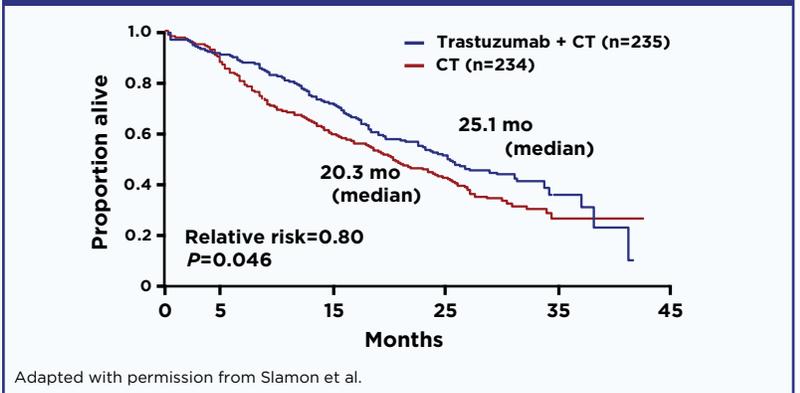
The pivotal trial that established the efficacy of trastuzumab combination regimens in the treatment of patients with HER2-overexpressing metastatic breast cancer compared responses in patients who received chemotherapy plus trastuzumab with those who received chemotherapy alone.³² For this study, chemotherapy consisted of an anthracycline plus cyclophosphamide for patients who had not received

prior anthracycline, or paclitaxel for patients who had received adjuvant anthracycline therapy. Analysis of the entire patient cohort, as well as the 2 chemotherapy treatment groups, showed that addition of trastuzumab to chemotherapy resulted in longer progression-free survival and longer overall survival (Figure 7³²), as well as a higher rate of response (50% vs 32% for chemotherapy alone; $P < 0.001$). In a subsequent study, patients with HER2+ metastatic cancer were randomly assigned to receive docetaxel (100 mg/m² every 3 weeks) with or without trastuzumab (a 4-mg/kg loading dose followed by 2 mg/kg weekly).³³ Trastuzumab plus docetaxel was significantly superior to docetaxel alone, in terms of response rate (61% vs 34%, respectively; $P = 0.0002$), overall survival ($P = 0.0325$), duration of response (median 11.7 vs 5.7 months, respectively; $P = 0.009$), and time to progression (TTP; median 11.7 vs 6.1 months, respectively; $P = 0.0001$) (Figure 8³³). In the TAnDEM study, postmenopausal women with HER2+ and ER- and/or PgR-positive metastatic breast cancer were randomly assigned to receive anastrozole plus trastuzumab or anastrozole alone.³⁴ Median progression-free survival was longer in the combination arm (4.8 months vs 2.4 months; $P = 0.0016$), as was TTP. However, no significant difference was seen in overall survival (28.5 months vs 23.9 months, respectively; $P = 0.325$). Data from these and other trials confirmed the efficacy and safety of trastuzumab plus chemotherapy in treating patients with HER2+ metastatic breast cancer.

Lapatinib Trial in First-Line Therapy

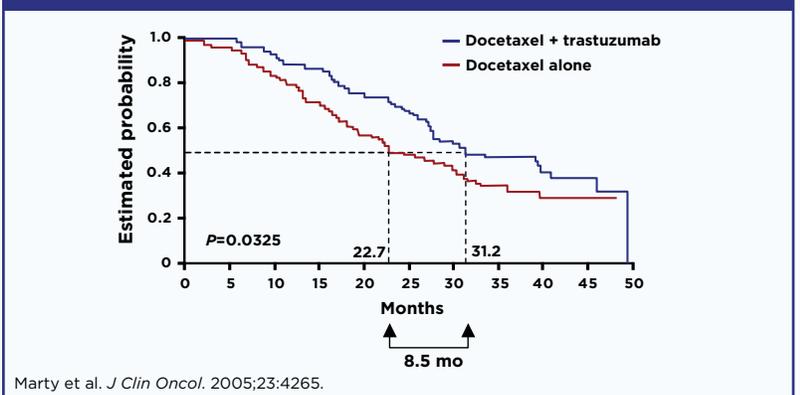
As mentioned previously, the second approved HER2-targeted agent that has demonstrated efficacy in HER2-overexpressing metastatic breast cancer is lapatinib, a tyrosine kinase inhibitor with specific activity against HER2. Lapatinib is currently approved, in combination with capecitabine, for the treatment of advanced or metastatic HER2+ breast cancer after prior therapy including an anthracycline, a taxane, and trastuzumab. A recent phase 3 trial evaluated the efficacy and safety of paclitaxel (175 mg/m² every 3 weeks) plus lapatinib (1500 mg/d) or placebo, in the first-line setting.³⁵ No clinically

Figure 7. Pivotal Combination Trial of First-Line Chemotherapy ± Trastuzumab in MBC: Overall Survival³²



significant benefit was demonstrated with the addition of lapatinib in the intent-to-treat population. However, a post hoc subgroup analysis of patients with HER+ breast cancer showed longer TTP in the lapatinib arm vs the placebo arm (median 36.4 vs 25.1 weeks; $P = 0.005$), as well as longer event-free survival (median 35.1 vs 21.9 weeks; $P = 0.004$) and increased response rate (63.3% vs 37.8%; $P = 0.023$). Because this was a post hoc analysis, caution must be taken in interpreting the data, but it is entirely consistent with the prior clinical and preclinical data suggesting that this drug's activity would be confined to HER2+ disease.³⁶ Another trial tested lapatinib combined with first-line aromatase inhibitor therapy (letrozole) in metastatic breast cancer. In this trial, patients with hormone receptor-positive breast cancer were randomly assigned to receive letrozole (2.5 mg/d) plus lapatinib (1500 mg/d) or letrozole plus placebo. Again, the clearest evidence for activity was in the HER2+ subset.³⁷

Figure 8. First-Line Randomized Phase 2 Trial of Docetaxel ± Trastuzumab in MBC: Survival

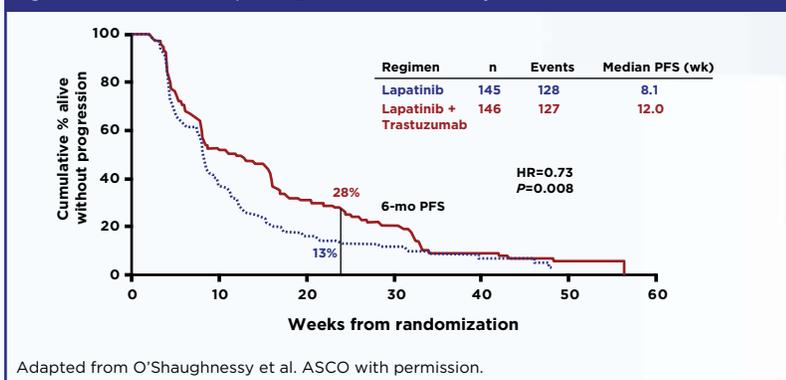


HER2-Targeted Agents After Progression on Trastuzumab

Despite the significant efficacy of trastuzumab in patients with HER2+ metastatic breast cancer, almost all patients will experience disease progression while on trastuzumab therapy. A number of clinical studies have been carried out to evaluate treatment strategies beyond progression on trastuzumab. In one study, patients who had progressed on trastuzumab were randomly assigned to receive capecitabine (2500 mg/m², days 1 to 14 of a 3-week cycle) with or without trastuzumab (6 mg/kg, every 3 weeks).³⁸ Although this study was closed before full patient accrual, treatment with the combination regimen resulted in a significant improvement vs capecitabine alone in TTP (8.2 vs 5.6 months; $P=0.0338$) and response rate (48.1% vs 27.0%; $P=0.0115$) but no significant change in overall survival (25.5 vs 20.4 months; $P=0.2570$). As described above, lapatinib also has demonstrated efficacy as a second-line agent in patients with HER2+ breast cancer, and trials have been carried out to assess the activity of lapatinib as monotherapy, or in combination with other agents in patients with HER2+ advanced or metastatic breast cancer, whose cancer has progressed on trastuzumab therapy. The study that led to the approval of lapatinib examined capecitabine (2000 mg/m²/d, days 1 to 14 of a 3-week cycle) plus lapatinib (1250 mg/d) vs capecitabine plus placebo and demonstrated an increase in median TTP (8.4 vs 4.4 months; $P<0.001$) and median progression-free survival (8.4 vs 4.1 months; $P<0.001$) for the lapatinib combination arm in patients with HER2+ metastatic or advanced breast cancer that had progressed after treatment with regimens that included an anthracycline, a taxane, and trastuzumab.³⁹

In studies of the efficacy of lapatinib monotherapy in patients who had disease progression on prior therapy, including chemotherapy and trastuzumab, modest activity was seen in patients with HER2+ disease.⁴⁰ In one study, a response rate of 4.3% (by investigator review, 1.4% by independent review) was achieved. A more recent study of lapatinib monotherapy (1250 or 1500 mg/d) in patients whose cancer had progressed

Figure 9. Phase 3 Trial of Lapatinib ± Trastuzumab in Heavily Pretreated HER2+ MBC: PFS⁴²



on trastuzumab therapy demonstrated the clinical benefit of lapatinib in this patient population.⁴¹ Clinical benefit rates (consisting of complete response, partial response, or stable disease for ≥ 24 weeks) were 14.1% by investigator review, and 9.0% by independent review.

In another study, patients whose disease had progressed on trastuzumab therapy were treated with lapatinib (1500 mg/d), or lapatinib (1000 mg/d) plus trastuzumab (4-mg/kg loading dose followed by 2-mg/kg doses every week).⁴² Improved efficacy was seen in the combination treatment arm, in terms of median progression-free survival (12.0 vs 8.1 weeks with lapatinib alone; $P=0.008$) (Figure 9⁴²). Median overall survival was 51.6 weeks in the combination arm, and 39.0 weeks in the lapatinib treatment arm ($P=0.106$). Response rates were not significantly different between the treatment arms, with a rate of 10.3% in the combination arm and 6.9% in the lapatinib arm ($P=0.46$). This speaks to the potential continued efficacy of trastuzumab despite prior progression on this agent.

HER2-TARGETED AGENTS IN THE ADJUVANT SETTING

The efficacy and safety of trastuzumab in the adjuvant setting has been assessed in a number of clinical trials. The National Surgical Adjuvant Breast and Bowel Project trial B-31 (NSABP B-31) and the North Central Cancer Treatment Group trial N9831 (NCCTG N9831) were designed to examine the efficacy of chemotherapy with or without trastuzumab in

patients with operable HER2+ breast cancer.⁴³ In the NSABP B-31 trial, patients were randomly assigned to receive doxorubicin (60 mg/m² every 3 weeks) and cyclophosphamide (600 mg/m² every 3 weeks), each for 4 cycles, followed by 175 mg/m² of paclitaxel every 3 weeks for 4 cycles, or the same regimen plus trastuzumab (4-mg/kg loading dose, given with the first dose of paclitaxel, followed by 2-mg/kg doses given every week for 51 weeks). In the NCCTG N9831 study, the same regimen of doxorubicin and cyclophosphamide was used, but it was followed instead by weekly paclitaxel (80 mg/m² every week for 12 weeks) in 1 treatment arm. Patients in a second treatment arm received the same chemotherapy regimen followed by trastuzumab (a loading dose of 4 mg/kg followed by 2-mg/kg weekly doses for 51 weeks). Patients in the third treatment arm received the same chemotherapy regimen plus trastuzumab (a loading dose of 4 mg/kg followed by 2-mg/kg weekly doses for 51 weeks) beginning with the first dose of paclitaxel. A joint analysis of these studies demonstrated a clinical benefit for patients in the trastuzumab treatment arms. Disease-free survival at 4 years was 85.3% in the trastuzumab arms vs 67.1% in the control arms ($P < 0.0001$). Overall survival at 4 years was 91.4% in the trastuzumab treatment arms vs 86.6% in the control arms ($P = 0.015$). Significant benefit was also seen in terms of time to recurrence, time to distant recurrence, and death from breast cancer.

The BCIRG 006 trial is a randomized study to compare 3 treatment regimens: doxorubicin (60 mg/m² every 3 weeks) plus cyclophosphamide (600 mg/m² every 3 weeks) for 4 cycles, followed by docetaxel (100 mg/m² every 3 weeks for 4 cycles) (AC→T); the same chemotherapy regimen followed by 1 year of trastuzumab (beginning with the first cycle of docetaxel) (AC→TH); or docetaxel (75 mg/m² every 3 weeks for 6 cycles) plus carboplatin (AUC6 every 3 weeks for 6 cycles) plus 1 year of trastuzumab (TCH).⁴⁴ A disease-free survival benefit was seen in both trastuzumab-containing treatment arms relative to the AC→T arm (AC→TH: hazard ratio [HR] 0.49; TCH: HR 0.61).

In the FinHer trial, women with node-positive or high-risk node-negative breast cancer were randomly assigned to receive docetaxel (100 mg/m² on day 1 of a 21-day cycle) or vinorelbine (25 mg/m², days 1, 8, and 15 of a 21-day cycle), followed by 3 cycles of 5-fluorouracil (600 mg/m²)/epirubicin (60 mg/m²)/cyclophosphamide (600 mg/m²) (FEC) on day 1 of a 21-day cycle (in both treatment arms).⁴⁵ Women who had verified HER2+ cancer were randomly assigned to receive trastuzumab (9 infusions administered at 1-week intervals, beginning on day 1 of the first docetaxel or vinorelbine cycle, a loading dose of 4 mg/kg, followed by subsequent doses of 2 mg/kg) or no trastuzumab. Trastuzumab was not given during FEC administration. An analysis of data from patients with HER2+ cancer showed that the addition of trastuzumab to these chemotherapy regimens provided clinical benefit in this patient group. Kaplan-Meier estimates of survival free of recurrence at 3 years were 89.3% in the trastuzumab arms and 77.6% in the no-trastuzumab arms, and estimates of overall survival at 3 years were 96.3% and 89.7%, respectively. An update of FinHer trial results was presented at the St. Gallen Oncology Conferences: Primary Therapy of Early Breast Cancer International Conference. At that time, it was reported that overall survival was improved by the addition of trastuzumab to the docetaxel treatment regimen or the vinorelbine treatment regimen.

The HERA trial compared 1 or 2 years of trastuzumab (8-mg/kg loading dose, followed by 6 mg/kg every 3 weeks) treatment with observation, in patients with HER2+ breast cancer who had received locoregional therapy and neoadjuvant or adjuvant chemotherapy.⁴⁶ Results for the observation group of patients and the 1-year trastuzumab treatment group of patients were reported at a median follow-up of 1 year.⁴⁶ Kaplan-Meier curves showed an estimated 2-year disease-free survival of 85.8% in the trastuzumab arm vs 77.4% in the observation arm ($P < 0.0001$), and a 2-year time to disease recurrence of 90.6% vs 82.8% ($P < 0.0001$). However, overall survival was not significantly different between the treatment groups.

In a trial with a similar design (PACS-04), patients with node-positive breast cancer were randomly assigned to receive adjuvant therapy as follows: 6 cycles of FEC (5-fluorouracil 500 mg/m²; epirubicin 100 mg/m²; cyclophosphamide 500 mg/m² every 3 weeks) or 6 cycles of ED (epirubicin 75 mg/m²; docetaxel 75 mg/m² every 3 weeks), followed by radiotherapy.⁴⁷ When HER2 status of the tumors became available, patients with HER2+ tumors were randomly assigned to receive 1 year of trastuzumab therapy (8-mg/kg loading dose followed by 6 mg/kg every 3 weeks) or observation only. At 4 years, disease-free survival was not significantly different between the trastuzumab and observation treatment arms.

Overall, these studies demonstrate a consistent efficacy for trastuzumab in the adjuvant setting, especially when it is administered concurrently with chemotherapy. The next-generation trial, ALTTO (NCT00490139), will assess the relative efficacies of trastuzumab, lapatinib, and trastuzumab plus lapatinib in the adjuvant setting. This study consists of 4 treatment arms: paclitaxel plus trastuzumab; paclitaxel plus lapatinib; paclitaxel plus trastuzumab for 12 weeks followed by a 6-week washout period, followed by lapatinib for 34 weeks; and paclitaxel plus lapatinib plus trastuzumab. All study participants must have received at least 4 cycles of an approved anthracycline-containing (neo-)adjuvant chemotherapy regimen. This study is currently recruiting patients.

HER2-TARGETED AGENTS IN THE NEOADJUVANT SETTING

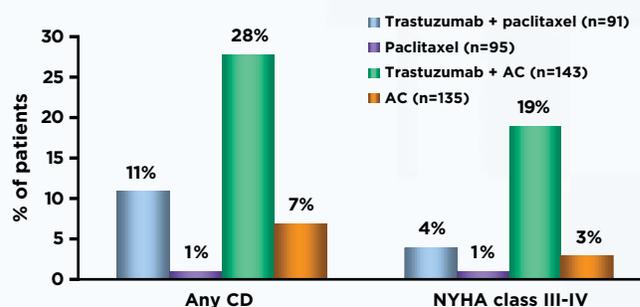
The use of HER2-targeted agents as part of a neoadjuvant regimen is less well-studied; however, encouraging data for HER2-targeted therapies have emerged in this treatment setting. A trial investigating the efficacy of neoadjuvant therapy with paclitaxel (225 mg/m² every 3 weeks for 4 cycles) followed by FEC (5-fluorouracil 500 mg/m² on days 1 and 4, epirubicin 75 mg/m² on day 1, cyclophosphamide 500 mg/m² on day 1) randomly assigned patients to receive trastuzumab (a loading dose of 4 mg/kg followed by weekly doses of 2 mg/kg, for a total of 24 doses of trastuzumab)

or no trastuzumab.⁴⁸ Pathologic complete response (pCR), generally a good correlate of progression-free and overall survival, was seen in 15 of 23 (65.2%) patients in the trastuzumab treatment arm, and in 5 of 19 (26.3%) patients in the no-trastuzumab arm. An updated analysis, which included data on additional patients, showed that 12 of 22 (54.5%) of the additional patients, treated with chemotherapy plus trastuzumab, achieved a pCR.⁴⁹

The NOAH trial assessed the efficacy of chemotherapy with or without trastuzumab in the neoadjuvant setting. Patients who had HER2+ disease were randomized to 1 of 2 treatment arms: doxorubicin (60 mg/m²) and paclitaxel (150 mg/m²) every 3 weeks, for 3 cycles, followed by 4 cycles of paclitaxel (175 mg/m² every 3 weeks) and 3 cycles of CMF (cyclophosphamide 600 mg/m², methotrexate 40 mg/m², 5-fluorouracil 600 mg/m² every 4 weeks, on days 1 and 8), followed by surgery and radiotherapy; or the same chemotherapy regimen, with trastuzumab (8-mg/kg loading dose followed by 6 mg/kg every 3 weeks for 1 year) for 1 year before surgery, and following surgery.⁵⁰ A pCR was achieved in 43% of patients in the trastuzumab arm vs 23% of those in the no-trastuzumab arm ($P=0.002$). Patients in the trastuzumab arm also benefitted in terms of event-free survival (HR 0.56) and overall survival (HR 0.65).

The Neo-ALTTO study (NCT00553358) is designed to assess the efficacies of lapatinib and trastuzumab in the neoadjuvant setting. In this study, patients will be randomized to 1 of 3 treatment arms: lapatinib followed

Figure 10. Pivotal Combination Phase 3 Trial of First-Line Chemotherapy ± Trastuzumab in MBC: Cardiac Safety



Slamon et al. *N Engl J Med.* 2001;344:783.

by lapatinib plus paclitaxel; trastuzumab followed by trastuzumab plus paclitaxel; lapatinib plus trastuzumab followed by lapatinib plus trastuzumab plus paclitaxel. This study is currently enrolling patients. The CALGB 40601 study (NCT00770809) also will assess the activities of trastuzumab and lapatinib in the neoadjuvant setting and will assign patients to 3 treatment arms: trastuzumab plus lapatinib plus paclitaxel; trastuzumab plus paclitaxel; or lapatinib plus paclitaxel. This study is currently recruiting participants.

HER2-TARGETED AGENTS: CARDIOTOXICITY

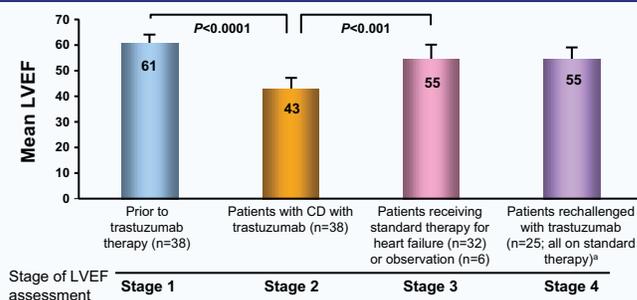
A key issue for HER2-targeted agents is that of cardiotoxicity. It was noted in the pivotal phase 3 trial of trastuzumab and in subsequent trials that a significant number of patients who received trastuzumab developed cardiac dysfunction, particularly when it was given concurrently with anthracyclines (Figure 10).³² Subsequent studies have shown that this cardiotoxicity is generally reversible and that trastuzumab may be reintroduced in some patients after resolution of the trastuzumab-induced cardiac dysfunction (Figure 11).⁵¹ An FDA review of cardiotoxicity in patients in 4 adjuvant breast cancer trials (NCCTG N9831, NSABP B-31, HERA, and BCIRG 006) concluded that there is a 4- to 6-fold increase in symptomatic myocardial dysfunction in patients in these trials who were treated with trastuzumab.⁵² It is recommended that patients who are candidates for trastuzumab therapy undergo a baseline assessment of risk for cardiac dysfunction, including a patient history, physical exam, and left ventricular ejection fraction (LVEF) assessment by multiple gated acquisition scan (MUGA) or echocardiogram. LVEF monitoring should be continued throughout the period of treatment with trastuzumab, although there is no evidence that such monitoring can prevent the development of symptomatic cardiac dysfunction. It is recommended that trastuzumab be withheld from patients with a $\geq 16\%$ absolute decrease in LVEF from pretreatment values, and from patients with an LVEF value below institutional limits of normal and a $\geq 10\%$ absolute decrease in LVEF from pretreatment values.

Cardiac safety has also been studied in patients who have been treated with lapatinib. A pooled analysis of data from 3689 patients in 44 clinical trials demonstrated that cardiac events occurred in a small percentage of patients (asymptomatic events in 1.4% of patients, symptomatic events in 0.2% of patients).⁵³ LVEF decreases were seen in a significant proportion of patients but, as seen with patients treated with trastuzumab, were often reversible. As with trastuzumab, LVEF assessment before beginning treatment with lapatinib, and throughout the treatment period, is recommended.

IV. FUTURE PIVOTAL TRIALS: NEW AGENTS AND NEW COMBINATIONS TO TARGET HER2

A number of clinical trials are under way to assess the activity of new HER2-targeted agents, and combinations of agents with lapatinib and trastuzumab. These agents include antibodies and specific protein inhibitors. Trastuzumab-DM1 (T-DM1), an antibody-drug conjugate that combines trastuzumab with DM1, a potent antimicrotubule agent, has shown activity in patients with HER2-overexpressing advanced or metastatic breast cancer in a number of studies (Table 1).⁵⁴⁻⁵⁶ Other antibodies in development include pertuzumab, a humanized monoclonal antibody that binds to the dimerization domain of HER2, which has shown promise in combination with trastuzumab or as monotherapy in patients whose breast cancer

Figure 11. Recovery From Trastuzumab-Related Cardiotoxicity⁵¹



• LVEF improved in most patients who stopped trastuzumab to receive therapy for heart failure, permitting trastuzumab re-treatment

*22/25 had no subsequent LVEF dysfunction.

Ewer et al. *J Clin Oncol*. 2005;23:7820.

Adapted with permission from Ewer et al.

CHALLENGES AND CONTROVERSIES

has progressed on trastuzumab therapy^{57,58}; and bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody.⁵⁹ Neratinib, an irreversible pan-HER receptor tyrosine kinase inhibitor, has demonstrated activity in combination with trastuzumab or as monotherapy.^{60,61} Pazopanib, an angiogenesis inhibitor that targets VEGF receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit, is being studied in combination with lapatinib⁶²; and tanespimycin, a heat shock protein 90 inhibitor, has shown clinical activity in combination with trastuzumab in patients whose cancer has progressed on trastuzumab therapy (Table 2⁵⁷⁻⁶³). Another agent that has shown promise is RAD001 (everolimus), a mammalian target of rapamycin (mTOR) inhibitor, which has been studied in combination with trastuzumab and other agents in patients with HER2+ metastatic breast cancer (Table 3^{64,65}). Much progress has been made in treating HER2-positive breast cancer, based on understanding the underlying biology of this malignancy. The rapid development of new agents based on this understanding suggests that treatment of this disease will continue to improve.

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Table 1. Trials of Novel Therapies Targeting HER2 in Breast Cancer: T-DM1⁵⁴⁻⁵⁶

Regimen	N	Dose	Safety	Efficacy
T-DM1 ⁵⁴	24 (15 pts received MTD)	0.3-4.8 mg/kg q3w MTD: 3.6 mg/kg	Grade ≥2 AEs infrequent; no grade 4 AEs reported at MTD; no cardiotoxicity reported	PR in 4 pts (44%) receiving MTD
T-DM1 ⁵⁵	27	1.2, 1.6, 2.0, 2.4, or 2.9 mg/kg qw	Dose-limiting toxicity in 2 pts at 2.9-mg/kg dose (thrombocytopenia); no other grade 4 AEs or cardiotoxicity	PR: 9/15 evaluable pts, 8 confirmed (53%)
T-DM1 ⁵⁶	112	3.6 mg/kg q3w	Most common grade 3/4 AE was thrombocytopenia, in 8 (7.0%) patients	ORR: 39.3% (42/107 evaluable pts) Confirmed ORR: 27.1% (29/107 evaluable pts)

Table 2. Trials of Novel Therapies Targeting HER2 in Breast Cancer⁵⁷⁻⁶³

Regimen	N	Dose	Safety	Efficacy
Pertuzumab/ trastuzumab ⁵⁷	66	420 mg q3w (840-mg loading dose) qw or q3w	Decrease in LVEF (<50% and ≥10%) in 3 pts; no pts withdrew due to cardiac AEs	CR: 7.6%; PR: 16.7%; SD 6 mo: 25.8%; SD <6 mo: 30%; PD: 50%
Pertuzumab ⁵⁸	29		Major adverse events: mild diarrhea and rash; no clinical cardiac events	Response in 2 patients with monotherapy; response in 2 patients upon addition of trastuzumab
Tanespimycin ⁶³	27	450 mg/m ² qw IV	Grade 3/4 AEs in 6 pts (fatigue, increased AST, and headache; n=2 each)	PR: 7/27 pts (26%)
Trastuzumab		qw		
HKI-272 (neratinib) ⁶⁰	136	240 mg qd	Grade 3/4 diarrhea in 30% of pts with prior trastuzumab, 13% of pts with no prior trastuzumab, 16% of pts had dose reduction due to diarrhea	ORR 26% in prior trastuzumab group, 56% in no prior trastuzumab group, 42% overall
Lapatinib ⁶²	141	Lapatinib 1500 mg/d (n=72), lapatinib 1000 mg/d + pazopanib 400 mg/d (n=69)	Grade 3/4 AEs in both arms: diarrhea, increased AST and ALT, vomiting; lapatinib arm: rash; lapatinib + pazopanib arm: nausea, fatigue, LVEF	Primary end point: PD rate at week 12: 28 (38.9%) in lapatinib arm, 25 (36.2%) in lapatinib + pazopanib arm
Trastuzumab ⁵⁹	50	Bevacizumab 10 mg on day 7, then q2w + trastuzumab qw	Most common AEs were fever, chills, headache, infusion reaction, 1 severe CE	ORR 54.1% (37 evaluable patients), CR 2.7%
Neratinib ⁶¹	45	Part 1: 160 mg or 240 mg qd Part 2: neratinib 240 mg qd + trastuzumab 4-mg/kg loading dose, then 2 mg/kg qw	Grade 3/4 AEs included diarrhea, nausea, vomiting. No AEs of CHF or significant drops in LVEF were reported	ORR 28.6% (8/28 evaluable pts), 16-week PFS rate 45%, median PFS 16 weeks

Table 3. Trials of Novel Therapies Targeting HER2 in Breast Cancer: mTOR Inhibitors^{64,65}

Regimen	N	Dose	Safety	Efficacy
Everolimus ⁶⁴	22	Everolimus 5-mg starting dose then 10 mg/d, or 30-mg starting dose then 50 mg qw or 70 mg qw	Febrile neutropenia in 1 pt, oral mucositis in 1 pt, confusional state in 1 pt	CR 5% (1/22 evaluable pts), PR 36% (8 pts), SD 50% (11 pts), CR + PR + SD 100% in 9 patients with taxane- and trastuzumab-resistant tumors
Paclitaxel Trastuzumab	(evaluable)			
Everolimus ⁶⁵	37	Everolimus 5 mg qd, or 20 mg qw, or 30 mg qw	DLTs: grade 3/4 neutropenia, grade 3 stomatitis, grade 3 fatigue, grade 2 dermatitis acneiform, grade 3 anorexia	CR 3% (1 of 34 evaluable pts), PR 15% (5/34), SD 62% (21/34)
Vinorelbine Trastuzumab				

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