Predictors of Resistance to Preoperative Trastuzumab and Vinorelbine for HER2-Positive Early Breast Cancer

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Abstract

Purpose: To assess pathologic complete response (pCR), clinical response, feasibility, safety, and potential predictors of response to preoperative trastuzumab plus vinorelbine in patients with operable, human epidermal growth factor receptor 2 (HER2) – positive breast cancer.

Experimental Design: Forty-eight patients received preoperative trastuzumab and vinorelbine weekly for 12 weeks. Single and multigene biomarker studies were done in an attempt to identify predictors of response.

Results: Eight of 40 (20%) patients achieved pCR (95% confidence interval, 9-36%). Of 9 additional patients recruited for protocol-defined toxicity analysis, 8 were evaluable; 42 of 48 (88%) patients had clinical response (16 patients, clinical complete response; 26 patients, clinical partial response). T1 tumors more frequently exhibited clinical complete response (P = 0.05) and showed a trend to exhibit pCR (P = 0.07). Five (13%) patients experienced grade 1 cardiac dysfunction during preoperative treatment. Neither HER2 nor estrogen receptor status changed significantly after exposure to trastuzumab and vinorelbine. RNA profiling identified three top-level clusters by unsupervised analysis. Tumors with extremes of response [pCR (n = 3) versus nonresponse (n = 3)] fell into separate groups by hierarchical clustering. No predictive genes were identified in pCR tumors. Nonresponding tumors were more likely to be T4 stage (P = 0.02) and express basal markers (P < 0.00001), growth factors, and growth factor receptors. Insulin-like growth factor-I receptor membrane expression was associated with a lower response rate (50% versus 97%; P = 0.001).

Conclusions: Preoperative trastuzumab plus vinorelbine is active and well tolerated in patients with HER2-positive, operable, stage II/III breast cancer. HER2-overexpressing tumors with a basal-like phenotype, or with expression of insulin-like growth factor-I receptor and other proteins involved in growth factor pathways, are more likely to be resistant to this regimen.

The importance of the human epidermal growth factor receptor 2 (HER2) and its effect on the course of breast cancer is widely recognized. The HER2 gene is amplified in 25% to 30% of breast tumors (1) and, in these patients, abnormally high levels of the encoded transmembrane glycoprotein are observed. HER2 overexpression is associated with an aggressive clinical course (2, 3), a shorter disease-free interval after adjuvant therapy, reduced response to hormonal therapy and non-anthracycline-containing chemotherapy (4, 5), and shortened overall survival (6, 7).

The development of the humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin, Genentech, Inc., San Francisco, CA) has altered the natural history of HER2-positive breast cancer. In the metastatic setting, treatment with trastuzumab is the standard of care (8, 9). Although trastuzumab is well tolerated, symptomatic cardiac dysfunction was reported in 16% of patients receiving concurrent trastuzumab and anthracycline-based chemotherapy in the pivotal metastatic breast cancer trials (10). Consequently, concurrent trastuzumab and anthracyclines are not indicated, and alternative combination regimens, based on cytotoxic drugs with a low risk of cardiotoxicity, have been investigated in this setting. There was a strong rationale for combining trastuzumab and vinorelbine for breast cancer therapy. Both agents have high single-agent activity and preclinical studies have suggested a synergistic effect (11, 12). In general, the two agents have nonoverlapping toxicity profiles (13). Although...
Multigene Profiling of Resistance to Trastuzumab

the biochemical mechanism for this interaction is not understood, HER2 receptor–mediated intracellular signaling may potentiate tumor cell sensitivity to vinorelbine by down-regulating survival signals (14). Trials done in patients with HER2-positive metastatic breast cancer showed encouraging response rates and minimal toxicity (15, 16).

The effectiveness of trastuzumab and chemotherapy in the metastatic setting prompted investigators to assess the efficacy and safety of adding trastuzumab to chemotherapy regimens for HER2-positive early breast cancer. Recent studies showed that adding trastuzumab to standard adjuvant chemotherapy enhanced disease-free and overall survival, with a <4% higher incidence of symptomatic cardiac dysfunction in the trastuzumab-containing treatment arms (17). The current study of preoperative vinorelbine and trastuzumab for HER2-positive stage II/III breast cancer was designed based on the results from trials assessing trastuzumab and vinorelbine as therapy for metastatic breast cancer and evidence of activity of trastuzumab and chemotherapy in the preoperative setting (18, 19).

Transcriptional profiling in human specimens has identified subclasses of breast cancer associated with different clinical outcomes (20–22). Among the categories emerging from these studies are estrogen receptor (ER)– and/or progesterone receptor (PgR)–positive tumors, HER2-positive tumors, and ER/PgR/HER2–negative tumors. Recent studies suggest that subsets of genes identified by transcriptional profiling can be used to predict response to therapy (23–26). Within the group of HER2-positive breast cancers, patterns of gene expression that are predictive of outcome with trastuzumab-containing treatment are likely to exist. In addition, profiling may identify genes associated with resistance to therapy previously undiscovered and suggest new treatment strategies. Single-gene biomarkers and multigene expression studies were done in an effort to identify predictors of response or resistance to trastuzumab and vinorelbine preoperative therapy.

Materials and Methods

Patient eligibility. Eligible patients were female or male, with stage II or III breast cancer (T ≥ 2 cm, N0–3, and M0), including patients with clinical T1N0M0 (pathologically confirmed nodal status), T2 lesions, and involved infracervical and supraclavicular lymph nodes. Patients with T1N0 tumors were not eligible. Patients were eligible based on HER2 overexpression by immunohistochemistry (immunohistochemistry 3+) and/or HER2 gene amplification by fluorescence in situ hybridization (FISH), done in a Clinical Laboratory Improvement Amendment–approved laboratory. Other eligibility criteria included the following: Eastern Cooperative Oncology Group performance status 0 or 1, age ≥ 18 years, left ventricular ejection fraction ≥ 50%, and adequate end organ function. Patients were ineligible if they had received prior trastuzumab, taxane, anthracycline, or vinorelbine therapy at any time. Patients were also ineligible if they were pregnant or nursing, had a history or symptoms diagnostic of systemic connective tissue or inflammatory disease, or had active or a history of severe cardiovascular or pulmonary disease. The Institutional Review Board–approved the protocol, and all patients provided written informed consent.

Treatment plan. After enrollment, all patients received preoperative treatment consisting of i.v. trastuzumab (4 mg/kg loading dose and then 2 mg/kg weekly for 11 weeks) and 25 mg/m² vinorelbine weekly for 12 weeks. Vinorelbine dose was reduced if absolute neutrophil count was <1,000/mm³ or platelet count was <99,000/mm³ and vinorelbine was delayed for treatment-related grade 3 nonhematologic toxicity.

Following surgery, the first eight patients received single-agent doxorubicin in a dose-dense fashion (60 mg/m² every 2 weeks for four cycles). To improve accrual, the protocol was amended to doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²; AC) every 3 weeks. After anthracycline-based therapy, patients received 12 doses of weekly paclitaxel (80 mg/m²) with concomitant trastuzumab (2 mg/kg) followed by every 3-week trastuzumab (6 mg/kg) to complete 52 weeks of trastuzumab. Due to the temporary closure of the concurrent paclitaxel and trastuzumab arm of the North Central Cancer Treatment Group N9831 adjuvant trial, the protocol was amended to allow optimal use of concordant chemotherapy in August 2002. Radiation and hormonal therapy were given at the discretion of the treating physician. Patients were withdrawn from the study if they showed a grade 4 nonhematologic toxicity, symptomatic congestive heart failure, progression during presurgical therapy, or treatment delay of >3 weeks for any toxicity-related reason.

Evaluation of tumor response and toxicity assessment. Pathologic complete response (pCR) was defined as the absence of invasive breast cancer in the breast and axillary lymph nodes at the time of surgery. Clinical tumor response was evaluated using Response Evaluation Criteria in Solid Tumors (27). Clinical complete response (cCR) was defined as no palpable tumor in the breast or lymph nodes before surgery. Clinical partial response (cPR), clinical stable disease (cSD), and clinical progressive disease (cPD) were defined using Response Evaluation Criteria in Solid Tumors. A verification measurement at least 4 weeks after cCR or cPR was not required. Cardiac function was measured by assessing left ventricular ejection fraction using multigated acquisition scan at baseline, before starting doxorubicin or AC, 3 months after doxorubicin or AC, and every 6 months thereafter for 2 years. Adverse events were graded according to National Cancer Institute common toxicity criteria version 2.0.

Biological assays: tissue biomarkers. HER2 status assessments by immunohistochemistry and FISH were done on the core biopsy before preoperative therapy and tissue at the time of surgery. ER, PgR, epidermal growth factor receptor (EGFR), and insulin-like growth factor-I (IGF-I) receptor (IGF-IR) assessments were also carried out at these time points. A central laboratory (Dana-Farber/Harvard Cancer Center Breast Pathology Core Laboratory) did correlative studies when tissue was available. The central laboratory HER2 and ER results were used for correlative studies. The following methods were used:

(a) HER2 by immunohistochemistry: HerceptTest (DakoCytomation, Carpinteria, CA) was done according to the manufacturer’s instructions. As defined in the HerceptTest kit guide, scores of 0 or 1+ were considered negative for HER2 overexpression, 2+ was a weak positive, and 3+ was a strong positive.

(b) HER2 by FISH: FISH (PathVysion, Vysis, Abbott Laboratories, Des Plaines, IL) was done on formalin-fixed, paraffin-embedded tissue sections by acid pretreatment and protease digestion followed by saline citrate buffer and formamide denaturation. Tissues were subsequently incubated with directly labeled fluorescent probes against HER2 (17q11.2-q12) and the pericentromeric region of chromosome 17 (CEP17: 17p11.1-q11.1). Spectrum green and orange signals were counted using fluorescence microscopy, 20 nuclei per section, and averaged and expressed as a ratio of HER2 copy number per chromosome 17 copy number. FISH was considered positive when more than two copies of CEP17 were present.

(c) ER assessment at the central laboratory: Following heat-induced epitope retrieval, paraffin-embedded tissue sections were incubated with the primary monoclonal anti-ER antibody clone 1D5 (1:400 dilution; DakoCytomation), secondary antibody, and peroxidase. After application of 3,3’-diaminobenzidine (Zymed, San Francisco, CA), slices were placed in 0.5% CuSO₄/0.9% NaCl for signal enhancement and then counterstained with hematoxylin; appropriate positive
and negative controls were used. ER expression was quantified by Allred score (28). This semiquantitative scoring system considers both the proportion of positive cells (scored on a 0-5 scale) and the staining intensity (scored on a 0-3 scale). The proportion and intensity scores are then added to produce total scores of 0 to 8. A score of >2 indicates ER positivity.

(d) EGFR assessment at the central laboratory: Following antigen retrieval, paraffin-embedded tissue sections were incubated with the antibody clone M3563 (1:200 dilution; DakoCytomation) and subsequently stained. EGFR staining was considered positive when tumor cells exhibited strong, circumferential membrane staining.

(e) IGF-IR assessment at the central laboratory: Following antigen retrieval, paraffin-embedded tissue sections were incubated with the antibody clone MS-641-P (1:100; NeoMarkers, Fremont, CA). IGF-IR staining was scored by study pathologists, and scoring was based on cellular location (cytoplasmic, membranous) and intensity [weak (1+), variable (2+), and strong (3+)]. A tumor was considered positive when tumor cells exhibited strong, circumferential membrane staining and was scored as 3+.

**Biological assays: transcriptional profiling.** Preoperative core biopsies were obtained using Biopsys Mammatome (Ethicon-Endosurgery, Johnson & Johnson, Cincinnati, OH); immediately frozen on dry ice, embedded in ornthine carbamyl transferase compound (TissueTek, Sakura, Torrance, CA), and stored at −80°C. Invasive carcinoma (>80%) was macrodissected from 7 μmol/L frozen tissue sections, and a minimum of 500 cells per case were used for RNA extraction. A total of 22 of 27 RNA samples could be successfully amplified using Nanoprep RNA kit (Stratagene, La Jolla, CA) and two rounds of RNA amplification. RNA was hybridized to GeneChip U133 Plus 2.0 Gene Arrays (Affymetrix, Santa Clara, CA). dChip software7 was used for analysis. Clustering algorithms were applied to a gene list of 3,133 genes identified using filtering criteria of >1.2-fold variation in expression; mean expression value difference was >100 (P < 0.001). Unsupervised hierarchical clustering and supervised analyses were done. Quality control measures included assessment of 3′ bias and quantitative real-time reverse transcription-PCR.

**Statistical methods.** The trial was designed as a two-stage study. In stage 1, 25 patients were accrued and, if at least two patients achieved a pCR, another 15 patients were to be enrolled, to a total of 40 patients. After the postsurgical treatment changed from doxorubicin to AC, the sample size was increased to 48 to evaluate the toxicity of trastuzumab.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics at baseline</th>
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<tr>
<td>FISH+**</td>
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<td>5-10 Copies/CEP17</td>
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<td>&gt;10 Copies/CEP17</td>
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<td>FISH−**</td>
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**Abbreviations:** ER+, estrogen receptor positive; PgR+, progesterone receptor positive; ER−, estrogen receptor negative; PgR−, progesterone receptor negative; IHC, immunohistochemistry; FISH+, FISH positive; FISH−, FISH negative.

*No grade on preoperative specimen.

**FISH** was done on the biopsy tissue in all cases with immunohistochemistry 0, 1+, or 2+ scores and in immunohistochemistry 3+ cases where tissue was available.

Between two or more available genes, with the assumption that the samples follow high dimensional Gaussian distribution and share the same covariance.

**Results**

**Patient population.** Forty-nine patients were enrolled onto the trial between June 2001 and May 2003; 1 patient was ineligible as the patient’s tumor was found to be HER2 negative on central testing. All 48 eligible patients received preoperative vinorelbine and trastuzumab. Following surgery, the first 8 patients enrolled received adjuvant single-agent doxorubicin every 2 weeks, and the remaining 40 patients received adjuvant AC every 3 weeks. Table 1 shows the baseline characteristics of the 48 eligible patients. A total of 24 (50%) patients had stage III disease. Twenty-eight patients had tumors that were characterized as grade 3, 15 had grade 2, 4 had grade 2/3
tumors, and 1 had no grading done on the preoperative specimen. The majority (85%) of patients had tumors that were strongly HER2 positive by immunohistochemistry (immunohistochemistry 3+). Patients with HER2 immunohistochemistry scores of 0 to 2+ were retested by FISH and all were gene amplified.

Per trial design, we report pCR data, modeling for pCR and cCR, and overall response on the first 40 of 48 eligible patients enrolled in the trial. All 48 patients were evaluable for clinical response, noncardiac toxicities, and biological assays.

**Clinical efficacy.** The study protocol permitted pCR analysis and step-up logistic regressions for the first 40 patients. A total of 8 (20%) patients achieved pCR (95% confidence interval, 9-36%). cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD. cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD. cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD. cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD. cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD.

All patients (3 of 3, 100%) with T1 tumors achieved cCR overall response rate was 85%; 34 of 40 patients had cPR or cSD. cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD. cCR was observed in 15 of 40 (38%) patients, and the overall response rate was 85%; 34 of 40 patients had cPR or cSD.

**Outcome.** Median follow-up since diagnosis was 2.6 years (range, 0.9-3.8 years). All patients received trastuzumab and vinorelbine before surgery and either doxorubicin (n = 8) or AC (n = 40) as adjuvant therapy following surgery. After completion of adjuvant doxorubicin or AC, 46 of 48 (96%) patients received 1 year of trastuzumab, and the majority received a taxane. Twenty-one of the 48 (44%) patients did not receive concomitant trastuzumab and taxane. Thirty of 48 (65%) patients relapsed; 2 of these patients had T4 disease at presentation and 1 died of her disease. The third patient had a T4 tumor and developed a local recurrence, with no evidence of distant failure. All three patients’ tumors were HER2 positive by FISH, two were ER/PgR negative, and one was ER/PgR positive.

**Biological assays: single-gene biomarkers.** Among the 48 patients, baseline HER2 status by immunohistochemistry was 3+ in the majority (85%) of tumors. There was no apparent variation in response to trastuzumab and vinorelbine between tumors with baseline immunohistochemistry 3+ or immunohistochemistry 2+/FISH-positive scores. To assess dynamic changes in HER2 with therapy, we measured biomarkers pre-trastuzumab and post-trastuzumab and vinorelbine in patients with less than a cCR and who had available tissue (n = 22). HER2 immunohistochemistry score did not change in the majority (16 of 18, 88%) of patients with baseline immunohistochemistry 3+ tumors with two becoming 1+. Tumors that were immunohistochemistry 2+ at baseline became immunohistochemistry 0 in two cases, and one case that was immunohistochemistry 0 at baseline was scored as immunohistochemistry 3+ at time of surgery. The latter case was FISH positive at diagnosis and likely represented a false-negative result in the initial biopsy. There was no trend for improved response according to number of HER2 gene copies (i.e., patients with two to five copies responded to trastuzumab plus vinorelbine equally well as those with more than five copies). HER2:CEP17 gene copy number was similar before and after therapy [median, 7.13 (range, 1.19-10.26) and 6.91 (range, 1.12-8.6), respectively].

To assess the effect of trastuzumab on ER expression and localization, we did ER staining in patients with available pretherapy and posttherapy tissue (n = 27; Supplementary Tables S1 and S2). ER scoring increased by ≥3 Allred points in four cases and decreased by ≥3 Allred points in one case; however, this resulted in a change in ER classification (positive to negative) in only one case. The remaining cases had post-trastuzumab Allred scores within 2 points of the pre-trastuzumab measurement. There was no observed change in subcellular localization of ER in pretherapy versus posttherapy specimens. PgR status was unchanged in pretreatment and posttreatment specimens (data not shown). Therefore, hormone receptor content and localization do not change under treatment with trastuzumab and vinorelbine.

This study also evaluated tyrosine kinase–linked cell surface receptors other than HER2. IGF-IR expression was assessed in 46 of the 48 cases. Scores of 1+ or 2+ were considered negative (Fig. 1A), whereas strong (3+ score) circumferential membrane staining (Fig. 1B) was considered positive. Cases considered positive for IGF-IR showed a lower likelihood of response to trastuzumab and vinorelbine than those with negative IGF-IR staining (50% versus 97%; P = 0.001) in this cohort, as shown in Fig. 1C. Using stringent criteria to define positivity for EGFR,

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**Table 2. Clinical response to neoadjuvant trastuzumab plus vinorelbine**

<table>
<thead>
<tr>
<th>Response</th>
<th>Patients (N = 48), n (%)</th>
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<tbody>
<tr>
<td>cCR + cPR</td>
<td>42 (88)</td>
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<tr>
<td>cCR</td>
<td>17 (36)</td>
</tr>
<tr>
<td>cPR</td>
<td>29 (52)</td>
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<tr>
<td>cSD</td>
<td>5 (10)</td>
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<tr>
<td>cPD</td>
<td>1 (2)</td>
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the receptor was positive in 1 of 22 (5%) cases tested, both pre-trastuzumab and post-trastuzumab and vinorelbine.

**Biological assays: transcriptional profiling.** To identify novel biomarkers of response and resistance to trastuzumab and vinorelbine, gene expression arrays were used (all array data are available at the caArray data portal). Twenty-two tumors were successfully amplified and yielded target RNA for hybridization to the arrays. Patient and tumor characteristics of samples with and without microarray data were not different, with the exception of ER/PgR status. Tumors that were ER and/or PgR positive were more frequent in the microarray data set compared with the tumors without microarray data ($P = 0.02$; Supplementary Table S3).

Unsupervised analyses determined if class distinctions were present in this group of HER2-amplified/HER2-overexpressing primary tumors. Three top-level clusters were observed (Fig. 2). ER, PgR, and HER2 gene copy number did not differ by cluster; however, tumor stage was not uniformly distributed between groups. Tumors classified as T4 were more frequent in a single top-level cluster ($P = 0.02$; Fig. 2). In addition to stage and biomarker expression, response to treatment was analyzed. The two patients with nonresponding tumors and one patient with relapse within 6 months of therapy fell in the same top-level cluster containing the majority of T4 tumors. All tumors that achieved a pCR fell into a separate top-level cluster.

To further evaluate this finding, we did supervised analyses comparing expression patterns between T4 and T2/3 tumors. Supervised clustering by T stage generated a list of differentially expressed genes (Fig. 3). Closer inspection uncovered genes associated with basal-like tumors. The basal-like group is composed of high-grade tumors that are both ER negative and HER2 negative (31). Further comparison of genes typically underexpressed or overexpressed in the basal-like subgroup from two independent data sets (20, 32) showed a highly significant correlation with genes, which were underexpressed and overexpressed in T4 tumors from this data set [$P < 0.00001$ (21) and $P < 0.00001$ (32)]. Twenty of 47 genes overexpressed in T4 tumors are characteristic of basal-like breast cancer.

Figure 3 shows genes overexpressed in T4 tumors, which include the basal keratins (CK14/15, CK5, and CK17), myoepithelial genes [$\gamma$-aminobutyric acid $\pi$ subunit (GABRP) and brother of CDO (BOC)], wnt family signaling genes (secreted frizzled and tent inhibitory factor), and other genes found consistently in the basal-like subtype [neuron navigator 2 (NAV2), tripartite motif 2 (TRIM2), NDRG family member, and nuclear factor I/B]. Several antiapoptotic genes (aB crystallin and clusterin) were overexpressed and found in basal-like tumors.

To explore gene expression patterns associated with response to treatment, supervised analysis was used to compare non-responding tumors with the remaining cohort for differentially expressed genes. Using pCR as the supervising term, we did not identify genes whose expression differed significantly ($P < 0.05$) in pCR tumors compared with the remaining group of tumors. For this analysis, we used several different normalization methods and filtering criteria. In contrast, resistant tumors (with lack of response or relapse within 6 months) did harbor genes whose expression differed significantly from the remaining tumors. Higher expression of several growth factors (hepatocyte growth factor, IGF-I, platelet-derived growth factor, and pleiotrophin), growth factor receptors (c-met and leptin receptor), the phosphatidylinositol 3-kinase regulatory subunit p85, and microtubule-associated protein 2 was observed. In addition, some basal genes were also expressed in this group (secreted frizzled-1, p63, and BOC). Moreover, T4 tumors that had not yet recurred clustered into the same branch of the dendrogram as nonresponding tumors, whereas there were no T2/3 tumors in this cluster.

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*a* http://caarraydb.nci.nih.gov/caarray/*
Although the inability to identify candidates for optimal response may be a function of the small numbers of samples with pCR \((n = 3)\), the same number of nonresponding tumors \((n = 3)\) was consistently and reproducibly associated with the genes noted above. Figure 4 depicts a linear discriminant analysis of pCR, resistant and remaining tumors, and shows that pCR tumors do not form a discrete cluster in three-dimensional gene space, whereas resistant tumors can be separated.

**Fig. 2.** Unsupervised hierarchical clustering of transcriptional profiles from tumors treated with trastuzumab and vinorelbine. Preoperative core biopsies \((n = 22)\) were macrodissected, and RNA was extracted, amplified, and hybridized to U133 Plus 2.0 Affymetrix GeneChips. Clustering and visualization were done with dCHIP software (http://www.dchip.org). Clinical variables depicted across the top of the figure include pathologic response phenotype \((\text{Path } \text{CR, pCR; } G, \text{ good/complete pCR; } B, \text{ bad/less than pCR})\), resistance phenotype \((\text{Clin Prog, cPD; } G, \text{ responsive to preoperative therapy; } B, \text{ no response; } R, \text{ relapse <6 mon from preoperative therapy})\), T stage \((2, 3, \text{ and } 4 \text{ per American Joint Committee on Cancer classification})\), ER expression \((P, \text{ positive; } N, \text{ negative})\), and PgR \((PRT)\) expression \((P, \text{ positive; } N, \text{ negative})\).

**Discussion**

This study shows that the combination of trastuzumab and vinorelbine is an active and well-tolerated preoperative therapy for patients with HER2-positive operable and locally advanced breast cancer. Two cases of grade 3 cardiac dysfunction that occurred after anthracycline adjuvant therapy proved reversible. The high activity and favorable safety profile of the regimen is
consistent with data from other studies evaluating the combination in metastatic breast cancer (15, 16), and the clinical responses are in accordance with prior results reported with both paclitaxel-trastuzumab and docetaxel-cisplatin-trastuzumab administered for 12 weeks in the preoperative setting (18, 33).

In the present study, the only clinical variables associated with response to treatment were small tumor size (T1) and age (<45 years). No correlation was found between biological markers (HER2 immunohistochemistry 2+ versus 3+, HER2 FISH positive versus FISH negative, HER2 copy number by FISH, and ER negative versus ER positive) and clinical/pathologic response to trastuzumab and vinorelbine. The lack of correlation between HER2 status (immunohistochemistry 3+ versus immunohistochemistry 2+ and FISH positive versus FISH negative) and response is in contrast to the findings of other investigators (31). However, the present trial included only cases that were immunohistochemistry 3+ and/or FISH positive; immunohistochemistry <3+/FISH-negative cases were not eligible. Hence, it seems that other molecular features or...
patient-related variables are responsible for differences in response to trastuzumab and vinorelbine in tumors with high levels of HER2 expression.

Several biomarkers were evaluated in paired samples of tissue pretreatment and posttreatment to assess the effect of trastuzumab and vinorelbine on their expression. In the majority of cases, tumor samples remained HER2 positive, suggesting that loss of HER2 receptor overexpression and/or amplification is not a common occurrence. This finding supports evidence from other studies that showed stability of the HER2 gene abnormality during progression of disease (34, 35). However, a recent study reported changes between preoperative and postoperative HER2 status as determined by FISH; 43% of FISH-positive tumors became FISH-negative tumors after preoperative therapy, although these results may have been influenced by high copy number chromosome polysomy (33). As this study had few immunohistochemistry 2+/FISH-positive cases, the findings by Hurley et al. may not be reflected in these results. Significant modulation of ER expression on treatment with trastuzumab and vinorelbine was not observed in the present study. However, this may relate to the technical differences between ER assay techniques used in this study compared with other studies (36). In addition, patients with residual disease after treatment may harbor resistant or inadequately treated cancers, potentially explaining the constant ER expression and localization.

An important modifier of HER2 activity in cells, EGFR, has been shown to amplify HER2 signaling through heterodimerization (37) and may be relevant for response to trastuzumab treatment (38). Whereas other studies report that EGFR expression ranges from 20% to 60% in breast cancer and coexpression with HER2 in 24% of cases (39, 40), EGFR expression was infrequent in this study (5%). Expression of EGFR as measured by immunohistochemistry is highly variable and dependent on methods and reagents. In fact, the receptor density of EGFR on most HER2-positive cells is probably below the sensitivity threshold of most antibodies using archival tissue (41). It is difficult to correlate EGFR expression with response to preoperative therapy because of the infrequent expression of EGFR seen in our cohort.

Both preclinical and limited clinical data suggest that the IGF-IR is associated with resistance to trastuzumab (42–44). IGF-IR is frequently overexpressed in breast tumors. The immunohistochemistry staining patterns for IGF-IR in this study showed that tumors with membrane IGF-IR expression were significantly less likely to respond to trastuzumab and vinorelbine than tumors with mainly cytoplasmic IGF-IR. Finding IGF-I gene expression by array and IGF-IR protein expression on the membrane in resistant tumors suggests the presence of an autocrine loop and supports the suggestion that IGF-IR mediates resistance to trastuzumab therapy.

Microarray profiling has identified breast tumor classes of biological significance (20–22). In our use of microarray profiling, extremes of response fell into separate classes when cases were segregated by unsupervised hierarchical clustering, suggesting that response to trastuzumab and vinorelbine may be determined by gene expression patterns. Attempts to identify genes associated with pCR were not fruitful, suggesting that this group of tumors may not represent a discrete molecular group. This observation is supported by recent data from Rouzier et al. (23) who evaluated 82 breast tumors treated with preoperative 5-fluorouracil, doxorubicin, and cyclophosphamide. In the Rouzier study, the authors were able to identify a gene signature for basal-like tumors, which

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**Fig. 4.** Linear discriminant analysis of gene expression profiles from preoperative core biopsies by response category. Green blocks, pCR; blue blocks, resistant; red blocks, all others.
achieved pCR (10 of 22) but were unable to identify such a signature in HER2-amplified tumors with pCR (9 of 20). Furthermore, they noted that HER2-amplified tumors that achieved pCR did not share any of the differentially expressed genes noted in the basal-like group.

In contrast to the lack of a robust signature in tumors achieving pCR, nonresponding tumors seemed to represent a particular tumor phenotype in this study. T4 tumors were more frequent in the nonresponding cluster and these tumors were more likely to express genes associated with the basal-like phenotype first described by Sorlie et al. (20) and Perou et al. (21), which includes higher expression of basal keratins (CK5, CK14, CK15, and CK17), myoepithelial genes (GABRP and BOC), and genes (NAV2, TRIM2, NDRG family member, and nuclear factor I/B) found consistently in the basal subtype. In addition to the overexpression of ‘basal genes’, T4 tumors were more likely to show lower expression of specific transcripts observed by Perou et al. (21) and Wang et al. (32) in the basal gene expression signature \((P < 0.00001)\). The ability to profile T4 tumors and generate a list of genes characteristic of another tumor subtype implies larger HER2-positive tumors in this study are not just sampled at a different time but are genetically different from the other tumors.

Nonresponding tumors expressed higher levels of growth factors (IGF-I, hepatocyte growth factor, platelet-derived growth factor, and pleiotrophin) and growth factor receptors (IGF-IR, leptin receptor, and c-met) in this study. The significance of this finding is unclear but is of interest as IGF-IR, c-met, and leptin receptors have all been found in breast tumors and associated with activation of both mitogen-activated protein kinase and Akt pathways, which are critical for HER2 signal transduction (14, 45–48). Their presence in tumors resistant to trastuzumab-containing therapy suggests that activation of parallel pathways may release tumors from dependence on HER2 for proliferation and survival.

An alternative explanation for the association of growth factor expression and resistance to trastuzumab-based therapy may be because several of these growth factors (IGF-I, hepatocyte growth factor, and platelet-derived growth factor) are important components of the tumor-stromal microenvironment (47, 49–51). In vitro models show that tumor-stromal interactions are able to induce multidrug resistance, perhaps by promotion of survival pathways (52), the induction of stromal proliferation by stromal cytokines (53), or a stromal barrier between tumor cells and the immune system (54, 55).

Finally, several genes associated with survival were seen in resistant tumors. The antiapoptotic genes αB crystallin, clusterin, and the phosphatidylinositol 3-kinase pathway have all been implicated in promoting cell survival, suggesting potential mechanisms of resistance in this subgroup (56, 57). A better understanding of these genes and pathways may allow development of additional approaches to the treatment of HER2-positive breast cancer.

In conclusion, combined preoperative trastuzumab and vinorelbine is a well-tolerated therapy with a high level of activity. Initial findings from biological assays indicate that down-regulation of HER2 or loss of gene amplification is uncommon after 12-week exposure to trastuzumab-containing therapy, suggesting that acquired resistance to trastuzumab does not involve selection of non-HER2–overexpressing clones. Transcriptional profiling identified several candidates for poor response to trastuzumab and vinorelbine therapy, most notably genes associated with basal-cell origin and IGF-IR pathway members. The use of molecular phenotyping for response to trastuzumab-containing therapy is an important area of ongoing research.

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References


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