

should be referred for consideration of liver transplantation as soon as possible after their first presentation with metabolic decompensation.

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HER2 Status and Benefit from Adjuvant Trastuzumab in Breast Cancer

TO THE EDITOR: Trastuzumab, an antibody against the protein product of the human epidermal growth factor receptor type 2 (*HER2*) gene, improves progression-free survival and overall survival when added to chemotherapy in patients with metastatic breast cancer.¹ Initial trials enrolled patients with tumors that had a staining intensity of 2+ or 3+ for *HER2* on immunohistochemical analysis, but in subsequent studies, the benefit was limited to tumors with *HER2* amplification as determined by fluorescence in situ hybridization (FISH). Trastuzumab also improves disease-free survival and overall survival in the adjuvant setting.²

On the basis of its mechanism of action, the *HER2* gene copy number was expected to predict benefit from adjuvant trastuzumab. National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-31, which compared standard chemotherapy of four cycles of doxorubicin and cyclophosphamide followed by four cycles of paclitaxel (ACT) with ACT plus trastuzumab (ACTH) in the adjuvant setting, provided an opportunity to test

this hypothesis.² Available tissue blocks were examined at a central site by means of a Food and Drug Administration–approved *HER2* FISH assay. We found no significant association between *HER2* copy number and benefit ($P=0.60$). Even patients with normal gene copy numbers appeared to benefit (relative risk for disease-free survival, 0.40; 95% confidence interval [CI], 0.18 to 0.89; $P=0.026$).

HER2 status according to immunohistochemical analysis with the use of Herceptest (Dako) was also determined at a central site. Tumors that were negative on FISH and had an immunohistochemical staining intensity of less than 3+ were defined as “central *HER2*-negative,” as in our previous report.³ Among the 1787 patients with follow-up data, 174 patients had breast cancers that were found to be central *HER2*-negative (9.7%), yet these patients also appeared to benefit from trastuzumab (relative risk for disease-free survival, 0.34; 95% CI, 0.14 to 0.80; $P=0.014$) (Table 1).

To address the technical problems inherent in

Table 1. Relative Risks of Disease Progression and Death among Patients in the ACTH Group as Compared with the ACT Group.*

End Point and Central HER2 Assay†	ACT <i>no. of events/total no. of events</i>	ACTH <i>no. of events/total no. of events</i>	Relative Risk (95% CI)	P Value	P Value for the Interaction
Disease progression					
HER2-positive	163/875	85/804	0.47 (0.37–0.62)	<0.001	0.47
HER2-negative	20/92	7/82	0.34 (0.14–0.80)	0.014	
Death					
HER2-positive	55/875	38/804	0.66 (0.43–0.99)	0.047	0.08
HER2-negative	10/92	1/82	0.08 (0.01–0.64)	0.017	

* The 95% confidence intervals (CI) and P values were adjusted according to the number of positive nodes and estrogen-receptor status from the univariate Cox proportional-hazards model for each subgroup in the National Surgical Adjuvant Breast and Bowel Project B-31 trial. ACT denotes doxorubicin, cyclophosphamide, and paclitaxel, and ACTH ACT plus trastuzumab.

† Central HER2 assay results were defined as negative if they were negative by both fluorescence in situ hybridization (PathVysion, Vysis) and immunohistochemical analysis (Herceptest, Dako) and were defined as positive if either test was positive.

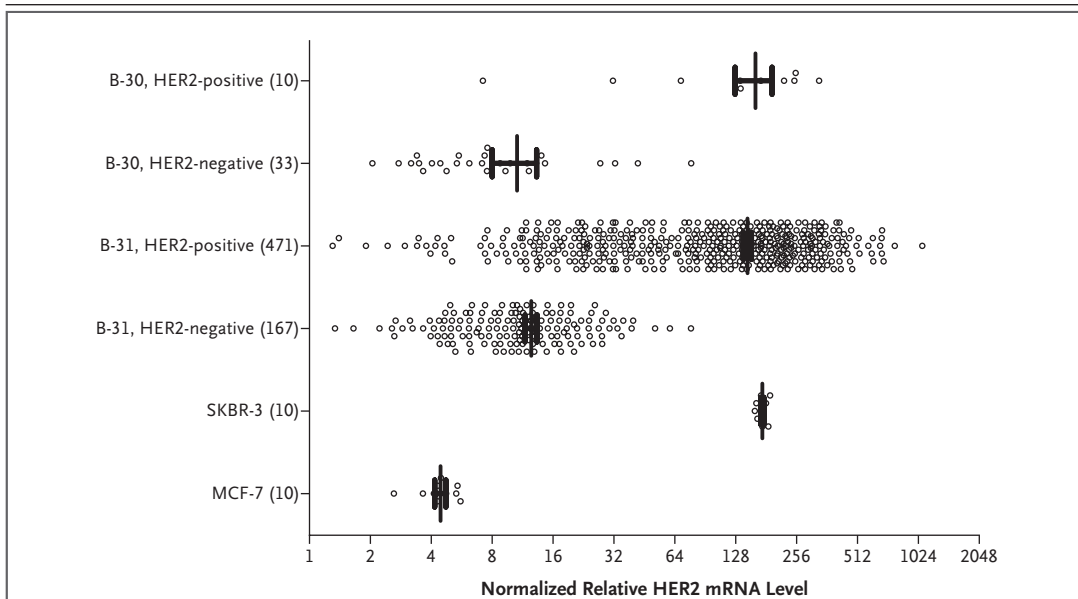


Figure 1. Scatter Plot of HER2 mRNA Levels in Each Category Defined by Centrally Performed HER2 Assays.

Each dot represents a measurement for each patient. Numbers of patients are shown in parentheses. Long vertical bars represent median values, and shorter vertical bars represent standard errors. The HER2 messenger RNA (mRNA) level was determined by a custom-designed Quantiplex branched-chain DNA amplification assay (Panomics). The HER2 hybridization signal was normalized to an average signal from five reference genes (*GAPDH*, *RPLPO*, *TFRC*, *GUSB*, and *ACTB*). SKBR-3 cells were used as positive controls, and MCF-7 cells were used as negative controls. Randomly selected samples from the National Surgical Adjuvant Breast and Bowel Project B-30 trial that were positive or negative for HER2 on the basis of fluorescence in situ hybridization were included for comparison.

current testing methods,^{3,4} we examined levels of expression of HER2 messenger RNA (mRNA) in a subgroup of 638 patients that included all 167 patients who had central HER2-negative tumors with enough tissue remaining in the blocks. The mRNA levels of HER2 in tumors that were central HER2-negative were significantly lower than those in HER2-positive tumors, and they were similar to those in HER2-negative tumors from another NSABP trial (B-30) (Fig. 1). The mRNA data provide strong evidence that the central HER2-negative tumors in the B-31 trial are indeed HER2-negative. Independent validation of the central FISH testing and immunohistochemical findings from the B-31 trial is being initiated.

Assuming that the validation studies are confirmatory, our findings suggest that the benefit of adjuvant trastuzumab may not be limited to patients with *HER2* amplification. Since our findings are based on an exploratory analysis, they should not alter current criteria used for selecting patients for adjuvant trastuzumab. Validation of the findings from central testing would justify a phase 3 trial of adjuvant trastuzumab in

women with breast cancers that do not meet established criteria for therapy.

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