Neoadjuvant Therapy with Paclitaxel followed by 5-Fluorouracil, Epirubicin, and Cyclophosphamide Chemotherapy and Concurrent Trastuzumab in Human Epidermal Growth Factor Receptor 2–Positive Operable Breast Cancer: An Update of the Initial Randomized Study Population and Data of Additional Patients Treated with the Same Regimen

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Abstract

Purpose: Findings from our previously published phase III randomized trial showed a high pathologic complete remission (CR) rate in patients with human epidermal growth factor receptor 2–positive breast cancer after the concurrent administration of trastuzumab and paclitaxel, followed by concurrent trastuzumab and 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) preoperative chemotherapy. The safety and efficacy data of initial population were updated, with inclusion of additional experience with the same therapy.

Study Design: The initial randomized study population of 42 patients were randomly assigned to either four cycles of paclitaxel followed by four cycles of FEC or to the same chemotherapy with simultaneous weekly trastuzumab for 24 weeks. All data were updated through November 2005.

Results: Pretreatment characteristics of the initial patients and of the second cohort were similar. In the second cohort, pathologic CR rate was 54.5% (95% confidence interval, 32.2-75.6%) and the pathologic CR rate among all patients treated with chemotherapy plus trastuzumab was 60% (95% confidence interval, 44.3-74.3%). Three patients in the chemotherapy only group have recurred, and one has died. There has been no recurrences in the patients randomized to chemotherapy plus trastuzumab, and the estimated disease-free survival at 1 and 3 years was 100% ($P = 0.041$). In additional cohort treated with chemotherapy and trastuzumab at the median follow-up of 16.3 months, no patients had recurred. No new safety concerns were observed in this study.

Conclusion: Our expanded cardiac safety data and the updated efficacy data showed that the natural history of this subset of breast cancer patients can be substantially modified by this treatment approach.

Findings from our previously published phase III randomized trial showed a high pathologic complete remission (CR) rate in patients with human epidermal growth factor receptor 2 (HER2)–positive breast cancer after the concurrent administration of trastuzumab and paclitaxel, followed by concurrent 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) preoperative chemotherapy (1).

Since these findings were published, the efficacy of trastuzumab as adjuvant therapy has been well established (2–4). In the adjuvant setting, trastuzumab has reduced the risk for recurrence and has favorably affected the survival rate in patients with HER2-positive breast cancer. In addition, findings in patients with metastatic disease and other experimental findings have suggested that the concomitant administration of trastuzumab and chemotherapy is superior to the sequential administration of these therapies (5–7). Preliminary unplanned data analyses of one prospective clinical trial (NCCTG-N9831) addressing the optimal timing of trastuzumab, in relation to chemotherapy, suggest the superiority of concomitant chemotherapy and trastuzumab over sequential use of the same therapies.[1] However, the optimal time to

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initiate trastuzumab therapy in relation to anthracycline-based chemotherapy remains to be defined. In our study, trastuzumab was concomitantly administered with chemotherapy to determine the effect of this approach on the pathologic CR rates.

To strengthen our earlier observations, we modified our original protocol to include additional patients. In this article, we report our further experience with concomitant administration of trastuzumab and paclitaxel followed by anthracycline-based preoperative chemotherapy in patients with HER2-positive breast cancer. We also expanded our previously published cardiac safety data and updated the safety and efficacy data from our initial study population.

Patients and Methods

Patients with histologically confirmed stage II to IIIA invasive but non-inflammatory carcinomas of the breast were included in this study. Fine-needle aspiration of clinically suspicious nodes was done, and all tumors were shown to be HER2/neu-positive by immunohistochemical or fluorescence *in situ* hybridization methods.

Details of the original study design were previously published (1). Briefly, initial study patients were randomized to receive either chemotherapy alone (paclitaxel followed by FEC therapy) or the same chemotherapy with trastuzumab weekly for 24 weeks. Before initiation of the study, each patient underwent a complete evaluation consisting of a medical history, physical examination, and staging to rule out metastatic disease. Cardiac evaluation included a baseline echocardiogram or multigated cardiac blood pool scan (MUGA). Patients with a history of uncompensated heart failure or of a cardiac ejection fraction of <45% were excluded. All patients were prospectively registered in our central research database. Each patient was informed about the investigational nature of this study, and the study protocol was approved by the institutional review board. Each patient signed a written informed consent before initiation of therapy.

We recorded tumor size after the first 12 weeks and after completion of all chemotherapy to determine the best clinical response before local therapy. Cardiac status was evaluated at baseline, after the completion of paclitaxel therapy, and again after the completion of FEC therapy. All patients were offered voluntary participation in a correlative science study that included a one-time pretreatment fine-needle biopsy of the cancer for gene expression profiling. Twenty-one patients participated in this biomarker discovery study. All 42 patients who participated in the original randomized phase of the study underwent serial blood sampling for troponin measurements in the blood and for proteomic response marker discovery.

At the completion of the original study, the protocol was amended to discontinue the chemotherapy alone arm and to add an additional 21 patients to the chemotherapy and trastuzumab arm. An additional cardiac evaluation in the follow-up period was included in the revised study to determine any delayed cardiac dysfunction following completion of therapy. The primary objective of this revised study was to estimate the efficacy of the chemotherapy and trastuzumab regimen. Specifically, it was estimated that 42 patients would provide a precision of 0.15 for the 95% confidence of pathologic CR. Pathologic CR was defined as no evidence of clinical invasive cancer in either the breast or axilla. There was no sample size justification based on the safety provided at the time of trial planning. However, if we assume that the true rate of cardiac toxicity is 5%, then we would expect to see at least one cardiac toxicity among the 42 patients with 88% probability.

Details of the chemotherapy we used were previously published (1). Briefly, each patient received four cycles of paclitaxel at 225 mg/m^2 as a 24-h infusion at 3-week intervals. Patients were then treated with four cycles of FEC therapy, which consisted of 500 mg/m^2 fluorouracil on days 1 and 4, 500 mg/m^2 i.v. cyclophosphamide on day 1 only, and 75 mg/m^2 epirubicin on day 1 only. The patients who had been randomized (in the original study) and assigned (additional study patients) to receive trastuzumab received 4 mg/kg trastuzumab i.v. over 90 min on day 1 of the first cycle of paclitaxel. These patients received 2 mg/kg trastuzumab weekly, administered i.v. over 30 min during the 24 weeks of chemotherapy.

Dose modification criteria were also previously reported (1). Briefly, the paclitaxel dose was reduced by 50% in subsequent cycles if a patient developed grade 3 neurotoxicity. Patients were required to have >1,500/μL granulocytes and >100,000/μL platelets before administration of the next cycle of chemotherapy. Pathologic specimens were evaluated for the presence of residual disease, and details of specimen evaluation were previously published. We updated the efficacy and safety data through November 2005.

Table 1. Patient characteristics

	No. patients		
	Randomized groups		Assigned treatment
	P + FEC alone	P + FEC + H	P + FEC + H
	(*n* = 19)	(*n* = 23)	(*n* = 22)
Age, y			
Median	48	52	51
Range	25-75	29-71	21-70
Tumor			
T_1	2	2	3
T_2	13	15	14
T_3	4	5	5
T_4	0	1	0
Nodal status			
N_0	7	10	9
N_1	12	12	13
N_2	0	1	0
Hormonal receptor status			
ER+ PR+	6	6	6
ER+ PR−	4	4	5
ER− PR+	1	3	1
ER− PR−	8	10	10
HER2 status			
FISH+	17	20	4
IHC 3+ only	1	3	1
IHC 3+ FISH−	1	0	0
IHC 3+ plus FISH+			17
Race			
White	13	13	14
African American	3	1	3
Asian	2	4	1
Hispanic	1	5	4

Abbreviations: ER, estrogen receptor; FISH, fluorescence *in situ* hybridization; H, trastuzumab; IHC, immunohistochemistry; P, paclitaxel; PR, progesterone receptor.

Results

The initial randomized study population of 42 patients was treated between June 2001 and October 2003, and the second cohort of 22 patients was treated between February 2004 and May 2005. One additional patient was enrolled in the second cohort with Institutional Review Board approval as this patient had already consented to participate in the study. Pretreatment characteristics of the initial randomized patients and of the second cohort assigned to receive chemotherapy and

trastuzumab are shown in Table 1. Patient distribution in both groups was similar with respect to age, tumor size, and nodal status between the groups. Approximately half of the patients had hormone receptor–positive disease.

Clinical response. The clinical response in our patients was assessed by physical examination of the breasts and nodes (Table 2). Most pathologic CRs were observed in patients who had clinical complete responses. A small number of patients clinically determined to have partial response or no change in their disease status had a pathologic CR. In the second cohort, the pathologic CR rate was 54.5% [95% confidence interval (95% CI), 32.2-75.6%], and the residual disease in an additional five patients consisted of only a cluster of a few tumor cells in the breast, with no residual disease in the nodes. Among the 45 patients who received chemotherapy plus trastuzumab, there were a total of 27 pathologic CRs and the pathologic CR rate was 60% (95% CI, 44.3-74.3%).

Safety data. No new safety concerns were observed in this study. In the initial study, the median left ventricular ejection fraction was 65% (range, 55-76%) in the chemotherapy alone arm, 65% (range, 50-71%) in the patients randomized to chemotherapy and trastuzumab, and 65% (range, 55-70%) in the second cohort treated with chemotherapy and trastuzumab; after 6 months of chemotherapy, these values were 65% (range, 55-70%), 60% (range, 52-70%), and 60% (range, 45-65%), respectively. Cardiac safety data are illustrated for the individual patients and for each treatment group over time in Fig. 1.

Among patients treated with chemotherapy alone, the left ventricular ejection fraction in one patient decreased to 35% following an acute myocardial infarction. In addition, this patient had a history of hypertension, diabetes mellitus, and mitral valve regurgitation. Among patients randomized to FEC and trastuzumab, the median left ventricular ejection fraction decreased to 60% by the end of follow-up, but the left ventricular ejection fraction range remained nearly constant. To date, patients in the initial cohort treated with trastuzumab have shown no clinical cardiac dysfunction. The follow-up period for the subsequent cohort of patients treated with chemotherapy and trastuzumab has been shorter than that for the randomized patients; however, the left ventricular ejection fraction median and range have decreased over time. In the second cohort, one patient had a history of atrial arrhythmias and left bundle branch block on her initial electrocardiogram. After completion of therapy, this patient had developed grade 1 cardiac dysfunction according to New York Heart Association Cardiac evaluation criteria and but has shown no further change in cardiac status during continued follow-up. None of the 45 patients treated with chemotherapy and trastuzumab in the initial and current study experienced clinical cardiac dysfunction, and there were no cardiac deaths in this study. The exact binomial 95% CI of the probability of cardiac failure was 0% to 7.87%.

Efficacy data. We estimated disease-free survival as measured from the date of study entry to the date of disease recurrence or last follow-up. No patients have died before developing disease recurrence. Figure 2 shows the disease-free survival curve for the randomized groups. The median follow-up among these patients was 36.1 months (range, 12.3-54.8 months). Three patients in the chemotherapy only (control) group developed recurrent disease, and among these, one patient died of progressive metastatic disease. Among the chemotherapy alone subgroup, disease-free survival at 1 year was 94.7% (95% CI, 85.2-100%) and at 3 years was 85.3% (95% CI, 67.6-100%). There has been no recurrent disease in the patients randomized to chemotherapy plus trastuzumab, and the estimated disease-free survival at both 1 and 3 years was 100% (1-year disease-free survival estimate: 95% CI, 85.2-100). Disease-free survival was significantly better among patients randomized to chemotherapy plus trastuzumab

Table 2. Extent of residual disease by treatment

	Randomized groups	Assigned treatment	
	P + FEC alone (*n* = 19)	P + FEC + H (*n* = 23)	P + FEC + H (*n* = 22)
pCR in breast and nodes, percentage (95% CI)	26.3 (9-51)	65.2 (43-84)	54.5 (32.2-75.6)
Residual disease in breast			
None	5	15	12
DCIS only in CRs	1	5	4
<1 cm	3	5	7*
1-3 cm	9	1	3
>3 cm	2	2	0
No. positive nodes			
0	15	20	20
1-3	2	3	2
4-10	2	0	
>10	0	0	
pCR by hormonal receptor status, no. patients			(*n* = 12)
Positive			6
Negative			6
			(*n* = 22)
No. segmental/sentinel biopsies			7
No. segmental/axillary dissections			7
No. modified radical/axillary dissections			8

Abbreviations: DCIS, ductal carcinoma *in situ*; pCR, pathologic complete remission.
*Focal cluster of cancer cells in 5 patients.

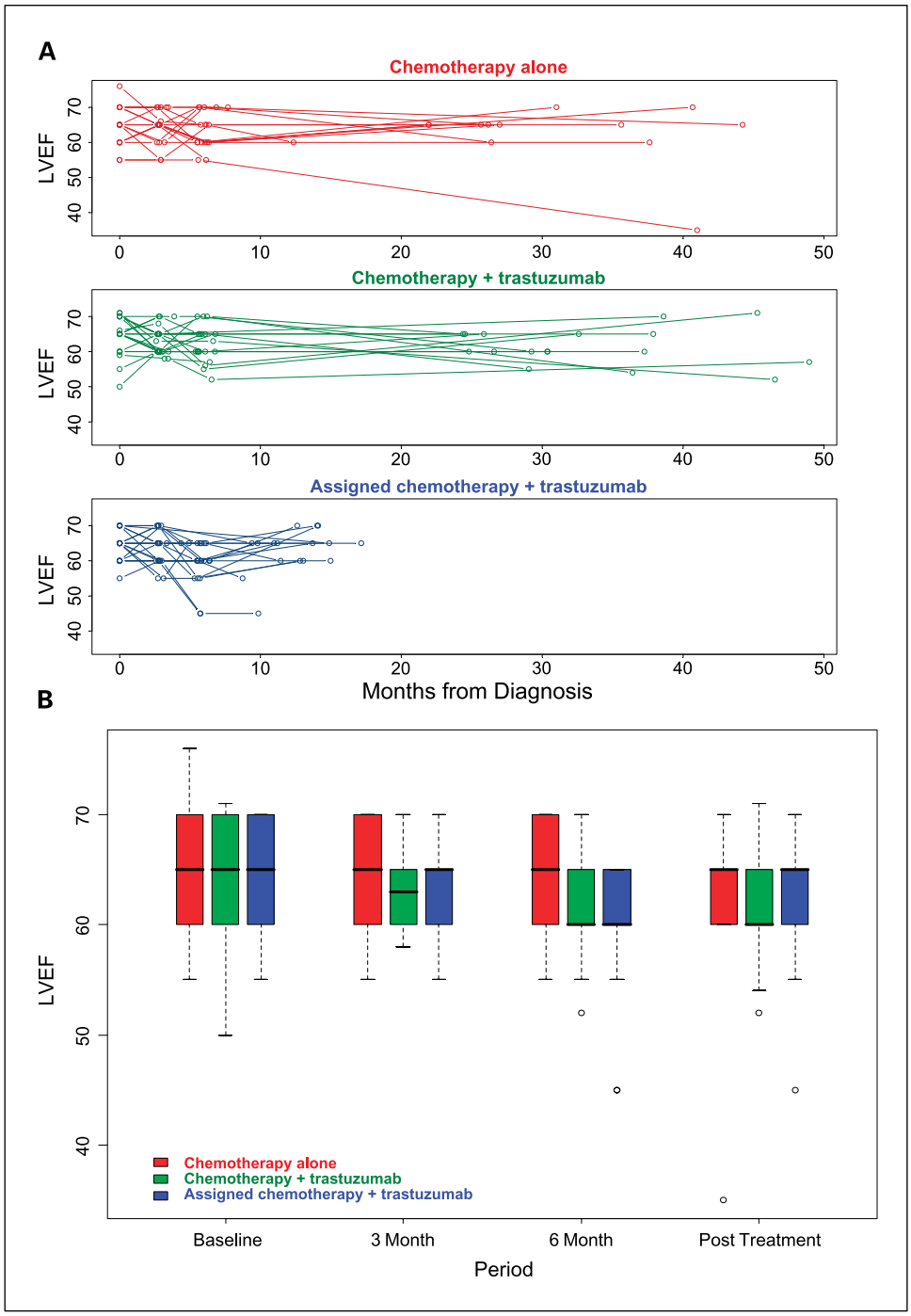

Fig. 1. *A,* ejection fraction data for individual patients by treatment over time. Each line represents an individual patient. *B,* ejection data for all patients. LVEF, left ventricular ejection fraction.

compared with patients randomized to chemotherapy alone (P = 0.041). Among the patients in the additional cohort treated with chemotherapy and trastuzumab, the median follow-up was 16.3 months (range, 5.9-20.4 months), and no patients had recurrent disease (1-year disease-free survival estimate: 95% CI, 83.9-100). In the combined two cohorts of patients treated with chemotherapy and trastuzumab, the 1-year 95% CI for disease-free survival was 92% to 100%.

Correlative science studies. The goal of the correlative science studies was to examine if molecular predictors of lack of complete response ("resistance") to concomitant trastuzumab and T/FEC preoperative chemotherapy can be identified through (*i*) gene expression analysis of pretreatment fine-needle biopsies of breast cancer or (*ii*) proteomic profiling of the plasma with matrix-assisted laser desorption time-of-flight technology. Gene expression profiling with Affymetrix U133A gene chips was done on 21 fine-needle aspiration specimens who participated in the original randomized clinical trial or in the single arm extension phase of the study and received preoperative trastuzumab therapy. Results from these studies were reported separately (8). Briefly, traditional clinical variables that predict pathologic CR to chemotherapy, including

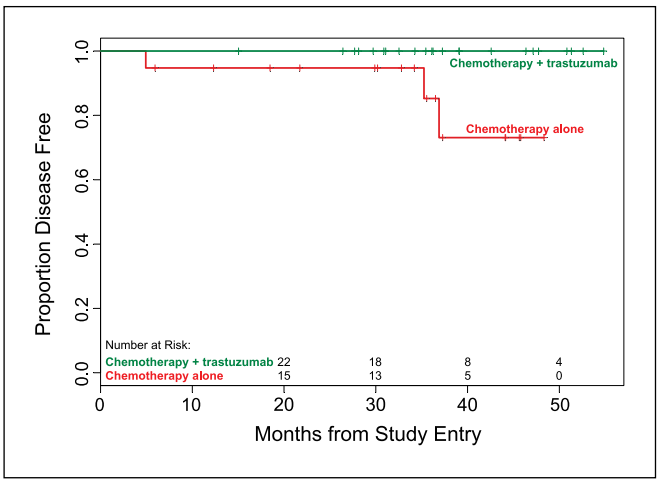

Fig. 2. Disease-free survival of randomized study population.

grade, ER status, or proliferative activity, lost their predictive value when trastuzumab was included in the treatment. HER2 mRNA levels or levels of gene amplification measured by fluorescence *in situ* hybridization also did not correlate with response. Analysis of gene expression data including 22,277 probe sets corresponding to >14,000 genes revealed no robust gene expression differences between cases with pathologic CR and those with residual disease. Gene Set Enrichment Analysis (9) suggests that increased expression of genes on chromosome 9q band 22 and decreased casapse expression may play a role in resistance to trastuzumab.

The proteomic analysis of the pretreatment serum samples is currently under way.

Discussion

Our findings, based on a cohort of patients treated for HER2-positive breast cancer in the same manner as patients in our earlier study, support our initial findings of high pathologic CR rates (1). Concurrent administration of trastuzumab and paclitaxel followed by anthracycline-based chemotherapy markedly enhanced these rates.

In addition, our expanded cardiac safety data and the updated safety and efficacy data from our initial study population showed that the natural history of this subset of breast cancer patients can be substantially modified by this treatment approach. To date, these efficacy data have been very encouraging because no patients in either group receiving chemotherapy or trastuzumab have developed recurrent disease. In contrast, three patients in the chemotherapy alone group developed metastatic disease. Patients who were followed up clinically and had a subsequent cardiac evaluation after completion of trastuzumab-based therapy have maintained their cardiac function in the same range, and no patients have developed clinically apparent congestive heart failure. These findings provide further evidence that trastuzumab and anthracycline-based combinations may be reasonably safe when used as in this protocol. Whereas some of the trastuzumab-treated patients have a decrease in their ejection fraction, these decreases have not progressed to clinical heart failure. This is in keeping with the concept that trastuzumab therapy is associated with type II treatment-related cardiac dysfunction that may be reversible and mechanistically or prognostically different than cardiotoxicity associated with anthracyclines (10). The long-term effect of trastuzumab on the heart has not yet been fully explored. The ejection fraction data for the individual patients suggest reversibility of trastuzumab-related cardiac dysfunction in some of our patients.

The optimal duration of trastuzumab therapy remains to be defined. In adjuvant trials, trastuzumab therapy was planned for 52 weeks. In our study, trastuzumab was planned to be administered concurrently with chemotherapy for only 24 weeks, and no further trastuzumab therapy was offered to any patient. Our small study compared favorably with the initially published data of the large adjuvant trastuzumab trial. In another small adjuvant study, trastuzumab administered concomitantly with the chemotherapy agents docetaxel or vinorelbine for a short duration substantially reduced the risk of recurrence (11). The Herceptin Adjuvant (HERA) trial is currently comparing the effects of 1 versus 2 years of trastuzumab adjuvant therapy and will provide pivotal information about prolonged trastuzumab adjuvant therapy (3).

In adjuvant trials, trastuzumab was given either after completion of all chemotherapy or after completion of the anthracycline phase of therapy. In a future prospective randomized trial of preoperative therapy, the optimal timing of the initiation of trastuzumab in relation to anthracycline- and taxane-based therapy will be evaluated. One arm of that study will be similar to the chemotherapy and trastuzumab arm of this study. Patients in the other arm will be treated initially with four cycles of anthracycline-containing chemotherapy alone (FEC) and then with trastuzumab and paclitaxel. A randomized study of preoperative therapy from our institution has shown higher pathologic CR rates with weekly administration of paclitaxel (12). Thus, paclitaxel in the upcoming trial will be given at 80 mg/m^2 per week because of these complete response rates and because of the safety profile of this dose.

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