

Pilot Trial of Trastuzumab Starting with or after the Doxorubicin Component of a Doxorubicin plus Paclitaxel Regimen for Women with HER2-Positive Advanced Breast Cancer

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ABSTRACT

Purpose: Combining trastuzumab with doxorubicin and paclitaxel (AT) is attractive because of the activity of AT and survival improvements observed when trastuzumab is added to either agent in HER2-positive metastatic breast cancer. This pilot study evaluates the efficacy and cardiac tolerability of AT followed by paclitaxel with trastuzumab started with AT or paclitaxel alone and investigates pharmacokinetic interactions.

Experimental Design: Two cohorts of 16 patients were enrolled. Cohort 1 received three cycles of AT (60/150 mg/m²) plus trastuzumab (4 mg/kg initial dose followed by 2 mg/kg), initiated concomitantly with doxorubicin, followed by nine cycles of paclitaxel (80 mg/m²) plus trastuzumab and then trastuzumab alone. Cohort 2 was treated with the same regimen, but trastuzumab was initiated with paclitaxel after AT. Cardiac function, pharmacokinetic interactions, and efficacy were evaluated.

Results: Median baseline left ventricular ejection fraction (LVEF) was 62% (range, 57–74%) and 66% (range, 57–77%) in cohorts 1 and 2, respectively. Most patients had an absolute decrease in LVEF. Congestive heart failure was not observed. LVEF in three patients decreased to <50% but recovered despite continued treatment. Response rates were 87.5% in both cohorts (cohort 1:2 complete response, 12 partial response; cohort 2:3 complete response, 11 partial response). No unexpected side effects were observed. Phar-

macokinetics of paclitaxel and its metabolites and of doxorubicin were similar without and with trastuzumab.

Conclusions: Trastuzumab administered with AT followed by weekly paclitaxel alone is highly active whether trastuzumab is initiated with AT or paclitaxel. Congestive heart failure was not observed, and LVEF decreases were reversible. Further studies of this regimen are warranted.

INTRODUCTION

Amplification/overexpression of HER2⁷ is associated with aggressive breast cancer and poor prognosis (1). Amplification occurs early in breast cancer, and experimental evidence indicates that it causes malignant transformation (2, 3). This stimulated investigation of anti-HER2 monoclonal antibodies as therapeutic agents and the eventual development of trastuzumab (Herceptin; Ref. 4). Trastuzumab is well tolerated and extends survival when administered first line with AC or paclitaxel to women with HER2-positive metastatic breast cancer (5–8).

Trastuzumab use, however, is associated with cardiotoxicity that resembles known anthracycline-related cardiotoxicity, particularly when used in combination with AC (8–10). This cardiotoxicity is generally manageable and reversible, often with continued trastuzumab therapy (11), although the combination of trastuzumab with AC is not approved for use outside clinical trials. Therefore, trials are ongoing to investigate ways of safely combining trastuzumab with less cardiotoxic anthracyclines, such as epirubicin and liposomal doxorubicin formulations or alternative doxorubicin schedules.

Doxorubicin and paclitaxel are widely used to treat breast cancer, and in combination they produce high response rates (12). The combination is associated with a higher than expected incidence of CHF above a cumulative doxorubicin dose of 480 mg/m² (13). Cardiac tolerability is similar to that of single-agent doxorubicin if the cumulative anthracycline dose is limited to 340–380 mg/m², continuing paclitaxel as a single-agent once this dose is reached (12, 14). The efficacy of AT therapy and the increased efficacy observed when trastuzumab is added to these agents suggest that it is rational to combine these three drugs.

This exploratory, within- and between-patient comparative, open-label trial was primarily designed to examine the acute tolerability of the combination using a low total dose of doxo-

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⁷ The abbreviations used are: HER2, human epidermal growth factor receptor-2; AC, doxorubicin/cyclophosphamide; CHF, congestive heart failure; IHC, immunohistochemistry; LVEF, left ventricular ejection fraction; CTC, Common Toxicity Criteria; TTP, time to progression; ECD, extracellular domain; C_{max}, maximum plasma concentration; AUC, area under the curve; NT-proBNP, N-terminal pro-brain natriuretic peptide; ORR, objective response rate; 95% CI, 95% confidence interval; CMF, cyclophosphamide-methotrexate-5-fluorouracil.

rubin (maximum of 180 mg/m²) and to explore pharmacokinetic interactions between trastuzumab, paclitaxel, and doxorubicin to determine whether this might explain the cardiotoxicity observed in previous trials. Secondary objectives were to investigate the feasibility, cardiac safety, and tolerability of AT followed by paclitaxel combined with trastuzumab administered either concomitantly with both components or with paclitaxel only.

MATERIALS AND METHODS

Study Design. Patients were enrolled in two cohorts of 16 patients. Cohort 1 received three cycles of AT plus trastuzumab, initiated concomitantly with doxorubicin, followed by nine cycles of paclitaxel plus trastuzumab and then trastuzumab alone. A second cohort of patients was treated with the same regimen, but trastuzumab was initiated with paclitaxel after AT (cohort 2).

Patients. Eligible patients were 18–69 years of age with stage IIIB/IV, measurable or evaluable breast cancer. HER2 overexpression was determined by IHC (DAKO HercepTest) in cohort 1, and a 2+ or 3+ score was required. Only 3+ overexpression or fluorescence *in situ* hybridization (Vysis) positivity was allowed for cohort 2 based on data from other studies. Other eligibility criteria included Eastern Cooperative Oncology Group performance status of 0 or 1; no previous chemotherapy for advanced breast cancer, although adjuvant chemotherapy (not including anthracyclines) was allowed; LVEF >50% and no history of myocardial infarction; and neutrophil or platelet counts >1,500/μL and >100,000/μL, respectively. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committees of the four participating institutions. Written informed consent was obtained from all patients.

Exclusion criteria included previous therapy with anthracyclines or trastuzumab; bone or central nervous system metastases as only disease site; creatinine or bilirubin levels >1.25 times the upper limit of normal and alanine or aspartate transaminase >1.5 times the upper limit of normal; uncontrolled hypertension; women who were pregnant, lactating or of child-bearing age not using adequate contraception; or enrollment in another investigational drug study. Concomitant therapy with any anticancer drug, including hormonal therapy, was not allowed. For cohort 2, patients were excluded if they had severe dyspnea at rest because of advanced malignancy or required supplemental oxygen.

All patients had a physical examination, liver and renal function tests, electrocardiography, echocardiography, determination of performance status, complete blood count, blood chemistry, and tumor assessment before treatment.

Therapy. AT was administered as an i.v. bolus of 60 mg/m² doxorubicin followed 15 min later by 150 mg/m² paclitaxel by infusion over 3 h. Both drugs were administered every 3 weeks for three cycles, followed by nine cycles of weekly paclitaxel (80 mg/m²) by infusion over 1 h. Patients received a paclitaxel premedication of i.v. dexamethasone (10 mg), i.v. diphenhydramine (50 mg), and i.v. cimetidine (300 mg) 30 min before administration of any active drug.

Trastuzumab was administered as a 4 mg/kg loading dose

followed by 2 mg/kg weekly. Treatment was initiated with the AT regimen in cohort 1: the first dose was administered as a 90-min infusion 24 h after AT in cycle 1; in cycles 2 and 3, trastuzumab was administered as a 30-min infusion followed by doxorubicin 15 min later and paclitaxel 15 min after the end of the doxorubicin infusion; in cycles 4–12, trastuzumab infusion was followed immediately by paclitaxel infusion. In cohort 2, trastuzumab was initiated with cycle 4 (first cycle of paclitaxel alone). Trastuzumab was administered until disease progression or 52 weeks of therapy but could be continued beyond 1 year until disease progression at the discretion of the investigator. Patients were followed for another 36 months.

Dose adjustment criteria for doxorubicin and paclitaxel were predefined. When administered in combination, doxorubicin and paclitaxel administration were delayed for 1 week in the event of granulocytes <1,500/μL or platelets <100,000/μL, and for an additional week if these counts persisted. The doxorubicin dose was reduced to 50 mg/m² in the event of grade 3 or 4 stomatitis or grade 2 stomatitis persisting at day 21; febrile neutropenia or infection of more than grade 2 and platelets <50,000/μL, granulocytes <1,000–1,499/μL, or platelets <100,000–150,000/μL after a 1-week delay, and after an additional 1-week delay. The paclitaxel dose was reduced to 150 mg/m² in the event of febrile neutropenia or infection of more than grade 2 and platelets <50,000/μL, granulocytes <1,000–1,499/μL, or platelets <100,000–150,000/μL after a one-week delay, and after an additional 1-week delay. The paclitaxel dose was reduced to 125 or 100 mg/m² in the event of grade 2 and 3 nonhematological toxicity, respectively, and was withdrawn in the event of grade 4 nonhematological toxicity. When paclitaxel was administered with trastuzumab after completion of the doxorubicin therapy, the dose was reduced to 70 or 60 mg/m² in the event of grade 2 and 3 nonhematological toxicity, respectively, and withdrawn in the event of grade 4 nonhematological toxicity. In the event of trastuzumab infusion-related events, subsequent trastuzumab infusions could be extended; if unacceptable toxicity occurred, trastuzumab was withdrawn.

Safety and Efficacy Evaluations. Adverse events were assessed weekly during cycles 1–3, once every 3 weeks during cycles 4–12, and once every 4 weeks thereafter. Their relationship to therapy was assessed by the investigator. Events were graded by use of the NCI-CTC.

Cardiac function was assessed by echocardiography every 3 weeks during chemotherapy (cycles 1–12) and every 4 weeks during trastuzumab monotherapy. Echocardiography data were reviewed centrally because of variations in methodologies between centers. Cardiac events were reported by use of the NCI-CTC. Cardiac events used to make decisions regarding whether to continue the trial were as follows: CHF, defined as S3 gallop, tachycardia, dyspnea, orthopnea, jugular venous distension, and/or edema consistent with CHF of NCI-CTC grade 3/4; three or more occurrences of cardiomyopathy (a global decrease in LVEF or more pronounced in the septum) or an asymptomatic LVEF decrease of >10% to <55% or any decrease to <50%.

Tumors were assessed after cycles 3 (week 9) and 12 (week 18), during week 26, and every 8 weeks thereafter. Response to therapy was assessed based on WHO criteria (15). TTP and overall survival were also assessed.

Pharmacokinetics, Cardiac Markers, and HER2 ECD

Assessment. The plasma pharmacokinetics of doxorubicin and paclitaxel were determined in cohort 1 during cycle 1 of AT treatment, when trastuzumab was delivered 24 h after chemotherapy, and during cycle 2, when patients received trastuzumab immediately before administration of bolus doxorubicin. An average of 21 blood samples for each cycle were collected over 72 h. Plasma doxorubicin, its major oxidative metabolite doxorubicinol, and the aglycone 7-deoxy-13-dihydro-doxorubicinone were measured by use of a previously described HPLC–fluorescence method characterized by online plasma sample extraction (16). Plasma paclitaxel and its main metabolite, 6 α -hydroxylpaclitaxel, were measured using UV-visible HPLC (17).

The C_{\max} was directly determined from analytical data. The $AUC_{0-24\text{ h}}$ was calculated by use of the log-trapezoidal rule. $AUC_{0-\infty}$ was determined by calculating $AUC_{0-\text{last}}$ and adding the area relative to the extrapolated region. The terminal half-life was derived by use of the formula: $t_{1/2} = 0.693/K_e$, where K_e is the slope of the linear equation that best fitted the last three or four concentration–time data points. Student's t test for paired data was used to evaluate statistical differences between pharmacokinetic parameters calculated in the absence (cycle 1) or presence of trastuzumab (cycle 2).

Serum trastuzumab and HER2 ECD levels were monitored over the duration of trastuzumab therapy: at baseline, at 3-week intervals during concomitant AT followed by paclitaxel plus trastuzumab, and at 4-week intervals thereafter. Analyses were conducted centrally using previously described methods (18, 19). Additional samples were drawn at baseline; at weeks 4, 7, 10, 13, 16, and 18; and every 4 weeks thereafter and tested for the cardiac markers troponin T and NT-proBNP with the Elecsys R Troponin T (Roche Diagnostics GmbH) and Elecsys R proBNP (Roche Diagnostics GmbH) kits, respectively.

Statistical Analysis. Because this was an exploratory study, no hypothesis was tested. All efficacy and safety analyses were descriptive and performed on the full data set. Overall ORRs and 95% confidence limits were calculated for each cohort. Kaplan–Meier estimates were used to calculate TTP (20). Safety data were summarized by use of descriptive statistics.

RESULTS

Patients and Treatment Received. Sixteen patients were enrolled into cohort 1 between October 1999 and May 2000 and 16 into cohort 2 between July 2000 and January 2001. Median duration of follow-up at present is 86.9 weeks in cohort 1 and 57.4 weeks in cohort 2. Baseline characteristics are shown in Table 1. The cohorts were generally comparable, although more patients in cohort 1 had HER2 2+ disease (seven *versus* none in cohort 2) because of the change in the protocol described above. Two and five patients in cohorts 1 and 2, respectively, had previous or existing cardiac disease that did not meet exclusion criteria, including tachycardia, aortic or mitral valve incompetence, and pericardial effusion. Three patients in cohort 1 and one in cohort 2 also had baseline cardiovascular risk factors (cohort 1:cardiac arrhythmia and hypertension, hyper-

Table 1 Patient characteristics at baseline

| Parameter | Cohort 1 (n = 16) | Cohort 2 (n = 16) |
|--|-------------------|-------------------|
| Mean age, years (range) | 49.7 (34–67) | 55.4 (25–66) |
| HER2 status (n) | | |
| IHC 2+ | 7 | 0 |
| IHC 3+ | 9 | 16 |
| ER ^a status (n) | | |
| Positive | 12 | 6 |
| Negative | 4 | 7 |
| Unknown | 0 | 3 |
| Disease stage (n) | | |
| IIIB | 6 | 4 |
| IV | 10 | 12 |
| Median no. of metastatic sites (range) | 2 (2–6) | 3 (1–6) |
| Disease site (n) | | |
| Regional node | 10 | 10 |
| Bone | 7 | 4 |
| Lung | 6 | 3 |
| Liver | 5 | 1 |
| Malignant pleural effusion | 5 | 1 |
| Distant node | 1 | 7 |
| Skin | 1 | 8 |
| Previous therapy (n) | | |
| Chemotherapy | 2 | 3 |
| Hormonal | 1 | 2 |
| Radiotherapy | 2 | 4 |

^a ER, estrogen receptor.

tension and hyperlipidemia, and hypertension in one patient each; cohort 2:diabetes mellitus and hypertension in one patient).

All patients were treated according to the protocol. Ten and 9 patients, respectively, completed 1 year of therapy in cohorts 1 and 2: 1 patient in cohort 1 withdrew because of disease progression during paclitaxel therapy (after cycle 10); 5 and 7 patients, respectively, withdrew during trastuzumab monotherapy because of progressive disease. Two patients in cohort 2 died of disease before week 52. One patient in cohort 1 received only one dose of doxorubicin because of a decrease in LVEF to <50%, and one patient in cohort 2 missed one cycle of paclitaxel because of nail toxicity. Median cumulative doses of doxorubicin and paclitaxel were 180 (60–180) mg/m² and 1170 (1010–1170) mg/m² in cohort 1 and 180 (all patients received three full-dose cycles) and 1170 (1090–1170) mg/m² in cohort 2. Overall, >95% of planned doxorubicin and >93% of planned paclitaxel doses were delivered at full dose. Median trastuzumab therapy duration was 52 (16–104) weeks and 42 (11–64) weeks in cohorts 1 and 2, respectively, reflecting the sequential enrollment of patients and initiation of trastuzumab at week 9 in cohort 2.

Cardiac Safety. Median baseline LVEF was 62% (range, 57–74%) and 66% (range, 57–77%) in cohorts 1 and 2, respectively; median 1-year values were 61% and 66%. The worst LVEF values during treatment and follow-up are shown in Table 2. Most patients in both cohorts had an absolute decrease in LVEF during the study. At cycle 12, LVEF decrease was observed in 75% and 33% of patients in cohorts 1 and 2, respectively.

Clinical CHF was not observed. However, the LVEF de-

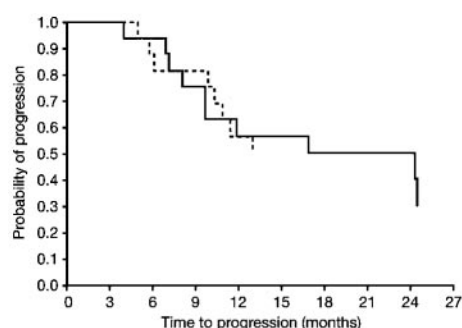


Fig. 1 Kaplan-Meier curve showing TTP in the two cohorts of patients. Solid line, cohort 1; dashed line, cohort 2.

creased from 58% to 40% after cycle 1 in one patient in cohort 1 who did not develop any symptoms. Doxorubicin was discontinued according to the protocol, but trastuzumab and paclitaxel were continued. The LVEF recovered to 56% at day 415 without cardiac therapy. Three other patients, all in cohort 1, developed asymptomatic LVEF decreases to $\leq 50\%$: decreases from 58% to 49% at cycle 3 and 43% at cycle 7; a decrease from 60% to 50% at cycle 7; and a decrease from 57% to 49% at cycle 10. Treatment was continued, and the LVEF recovered to above baseline without cardiac therapy in all three patients. No patient in cohort 2 had a decrease in LVEF to $< 50\%$, and six showed an increase or no change in LVEF. Troponin T, NT-proBNP, and proBNP concentrations were within the normal ranges and generally stable over time in both cohorts (data not shown). There was no relationship with decreases in cardiac contractility.

General Safety. Therapy was generally well tolerated. Events were typical of the drugs used, and the majority (89% and 93.8% in cohorts 1 and 2, respectively) were NCI-CTC grade 1 or 2 in severity. Nonhematological events, biochemical changes, and hematological abnormalities, excluding the already described cardiac events, occurred at a similar incidence in both cohorts. Only two NCI-CTC grade 4 events were observed, both in cohort 1 (diarrhea and malignant melanoma). The most frequent NCI-CTC grade 3/4 nonhematological events were amenorrhea (four and three events in cohorts 1 and 2, respectively), infection (three and no events in cohorts 1 and 2, respectively), and hypertension (one event in each cohort). The majority of patients in both cohorts experienced hematological abnormalities, mostly of NCI-CTC grade 1 or 2 (cohort 1: anemia, 100% grade 1/2 and 0% grade 3/4; neutropenia, 31% and 32%; leukopenia, 63% and 13%; cohort 2: anemia, 88% grade 1/2 and 0% grade 3/4; neutropenia, 44% and 12%; leukopenia, 63% and 12%).

No patient withdrew from the trial because of an adverse event, but in addition to the patient described above in whom doxorubicin was withdrawn after one cycle, the doxorubicin dose was reduced for neutropenia in one patient and the paclitaxel dose for nail abnormality and paresthesia in one patient each, respectively. Trastuzumab was withheld for 2 weeks and 4 weeks, respectively, for flu-like illness and deep vein thrombosis in one patient each in cohort 1. In addition, 1 week each of paclitaxel and trastuzumab was withheld for a nail abnormal-

ity and 2 weeks of trastuzumab were withheld for peripheral ischemia in one patient each in cohort 2.

Efficacy. Fourteen patients in each cohort responded to therapy (cohort 1: 2 with complete response, 12 with partial response, 2 stable disease; cohort 2: 3 with complete response, 11 with partial response, 2 with stable disease), for an ORR of 87.5% (95% CI, 61.65–98.45%). Five patients in each cohort had stage IIIB disease and underwent mastectomy followed by adjuvant CMF and radiotherapy at the end of study treatment. Both patients in cohort 1 who had stable disease as their best response had HER2 2+ disease. Time to response was a median of 2 months (range, 2–4 and 2–12 months in cohorts 1 and 2, respectively); median response duration was 16 (range, 5–23) months in cohort 1 and 10 (1–15) months in cohort 2. Median TTP was 20.4 months in cohort 1 and 12.8 months in cohort 2 (Fig. 1). Differences in median response duration and median TTP between the cohorts are largely attributable to the sequential enrollment, resulting in different observation periods (median pf 608 and 402 days, respectively). TTP showed a similar trend in the two cohorts over the interval when a comparison could be made (Fig. 1).

One-year survival rates were 100% and 62.5% in cohorts 1 and 2, respectively. Although there were two deaths in cohort 2 during the first year, no data were available at 1 year for four patients, which accounts for much of the apparent difference. This difference should be interpreted with caution given the few patients in each cohort, the difference in HER2 status of the cohorts, and the difference in duration of follow-up. Median overall survival data are not available because 29 of 32 patients are still alive.

Pharmacokinetics. The pharmacokinetic data for doxorubicin and paclitaxel and their metabolites are shown in Table 3. For paclitaxel, its major metabolite 6- α -hydroxypaclitaxel, and doxorubicin, the main pharmacokinetic measurements were similar with and without trastuzumab. However, exposure to doxorubicin metabolites was significantly greater in the presence of trastuzumab. For doxorubicinol, the difference was limited to the AUC, with an average increase of $\sim 12\%$ in the presence of trastuzumab. For the aglycone of doxorubicinol, the

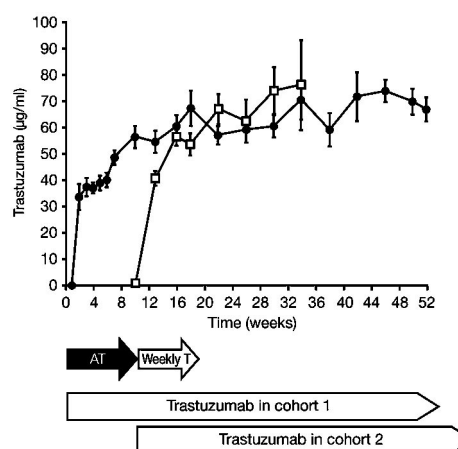


Fig. 2 Mean trastuzumab serum trough concentrations for patients in cohorts 1 (●) and 2 (□). Bars, SE.

Table 2 Worst LVEF values during treatment and follow-up based on central review of echocardiography data

| Cohort | Increase or no change | Change in LVEF (n) | | | Absolute value <50% |
|------------|-----------------------|--------------------|------|-------------|---------------------|
| | | Decrease | | | |
| | | <10% | ≥10% | ≥10 to <55% | |
| 1 (n = 16) | 0 | 4 | 4 | 4 | 4 |
| 2 (n = 16) | 6 | 4 | 5 | 1 | 0 |

Table 3 Pharmacokinetic data for doxorubicin and paclitaxel and their metabolites

| Drug | AUC | | | C_{max} | | | $t_{1/2}$ | | |
|-----------------------------------|------------------------|--------|---------------------|-----------|--------|-------------------|-----------|--------|------|
| | Without T ^a | With T | P | Without T | With T | P | Without T | With T | P |
| Doxorubicin | 1.29 | 1.44 | 0.10 | 3.37 | 3.86 | 0.24 | 43.11 | 33.80 | 0.09 |
| Doxorubicinol | 0.41 | 0.46 | 0.04 ^b | 0.03 | 0.04 | 0.24 | 47.84 | 40.75 | 0.16 |
| 7-Deoxy-13-dihydro-doxorubicinone | 0.16 | 0.24 | 0.0001 ^b | 0.01 | 0.02 | 0.03 ^b | 19.79 | 21.52 | 0.77 |
| Paclitaxel | 13.49 | 13.13 | 0.71 | 4.46 | 4.21 | 0.49 | 20.79 | 21.2 | 0.90 |
| 6-α Hydroxyl paclitaxel | 0.9 | 1.0 | 0.4 | 0.42 | 0.46 | 0.46 | 1.59 | 1.82 | 0.71 |

^a T, trastuzumab.

^b Significant difference.

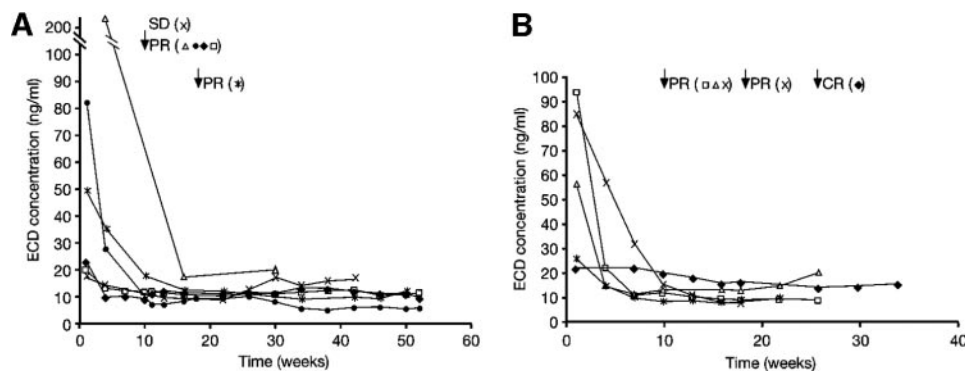


Fig. 3 HER2 ECD levels over time in relation to response to therapy in cohorts 1 (A) and 2 (B). Time of occurrence of best response is indicated.

average difference was ~50% for both AUC and C_{max} (Table 3). Trastuzumab serum trough concentrations were similar and as expected in the two cohorts (Fig. 2 Ref. 21).

HER2 ECD Levels. Baseline HER2 ECD levels were elevated (>15 ng/ml) in six and five patients in cohorts 1 and 2, respectively. Of these 11 patients, 10 achieved a clinical response, and HER2 ECD levels decreased rapidly to normal in all of these patients (Fig. 3). One patient, who had an initial HER2 ECD measurement of 17 ng/ml that initially decreased but later increased, had stable disease as her best response.

DISCUSSION

This trial was designed to assess the feasibility of combining trastuzumab with AT followed by paclitaxel. Special consideration was given to prospective evaluation of the cardiac tolerability of the combination and analysis of the plasma disposition of doxorubicin and paclitaxel in the absence and presence of trastuzumab to evaluate possible pharmacokinetic interactions. The data demonstrate the feasibility of combining AT

followed by paclitaxel with trastuzumab, the absence of symptomatic cardiac events, albeit in a small study population, and minimal although statistically significant increases in plasma exposure to doxorubicin metabolites in the presence of trastuzumab.

Selection of AT followed by paclitaxel was based on the excellent antitumor activity of AT and data indicating that continuous administration of single-agent paclitaxel after discontinuation of doxorubicin can increase complete response rates in patients with metastatic breast cancer (13, 22). In addition, trastuzumab improves the activity of both drugs in patients with HER2-positive disease (8). The emphasis of this trial was on the cardiotoxicity of the combination. The first report on the AT combination indicated a higher than expected incidence of CHF (20%; Ref. 13). This effect can be attributed to two main factors: paclitaxel, probably because of its formulation in cremophor EL, causes an almost 30% increase in doxorubicin exposure (16). In addition, similarly to docetaxel, paclitaxel promotes the metabolism of doxorubicin to cardio-

toxic species in human myocardium (23). Nevertheless, if AT is administered up to a maximum cumulative doxorubicin dose of 340–380 mg/m², the toxicity of the combination is similar to that of doxorubicin monotherapy (14). The tolerability of this approach has recently been confirmed in >600 women undergoing adjuvant/neoadjuvant therapy with AT followed by CMF (24).

Another reason for the aggressive cardiac monitoring was cardiotoxicity observed in trials of trastuzumab (11). Present hypotheses on the mechanism of trastuzumab-associated cardiotoxicity focus on exacerbation of preexisting anthracycline damage and the existence of a direct mechanism via the HER2 receptor. In support of the former, rates of cardiotoxicity appear lower and the syndrome less severe when trastuzumab administration is temporally separated from anthracycline exposure (9); cardiotoxicity is uncommon in women not exposed to anthracyclines before trastuzumab therapy (11). In addition, recent data indicate that the cardiac ultrastructural changes characteristic of anthracycline-related cardiotoxicity are not observed with trastuzumab, cardiac dysfunction is transient, and recovery is better than expected with standard therapy (25). Thus, the mechanism of cardiac damage could be similar to myocardial stunning (26). In the present study, decreases in LVEF in cohort 1 led to a second cohort being treated with trastuzumab only after completing doxorubicin therapy. Most patients in both cohorts had LVEF decreases. However, LVEF decreases to <50% occurred only in patients in cohort 1. Interestingly, LVEF recovered to normal levels with continued trastuzumab therapy in all patients experiencing decreases. This is consistent with previous reports indicating that the event is generally manageable, and contrasts with many reports of doxorubicin-induced cardiotoxicity, which indicated that prognosis is poor (10). However, results observed in recent studies on the effect of anthracyclines on cardiac contractility that incorporated aggressive prospective evaluation of patients requiring discontinuation of anthracyclines, continuous monitoring of patients, and cardiac medication when moderate alterations persisted indicated that cardiotoxicity may be manageable and devoid of serious consequences in most patients (27).

Data for cardiac markers failed to show any relationship with changes in cardiac function. However, the small numbers of patients in the study and the absence of clinical cardiac events limit this analysis. Thus, whether cardiac markers are a significantly more sensitive tool than LVEF in predicting symptomatic cardiac alterations awaits more in-depth, prospective analysis, which is now ongoing in at least two trials of adjuvant trastuzumab therapy.

The cardiac tolerability of the combination agrees with the findings of the pharmacokinetic investigation, in which trastuzumab was shown not to affect the plasma pharmacokinetics of the two cytotoxic drugs. However, exposure to the doxorubicin metabolites doxorubicinol and 7-deoxy-13-dihydro-doxorubicinone was increased in the presence of trastuzumab. Research has suggested that increased intramyocardial exposure to doxorubicinol plays a pivotal role in the pathogenesis of classical anthracycline cardiotoxicity (23, 28). However, the clinical significance of the increase in exposure to doxorubicinol in the presence of trastuzumab is likely to be minor, if any, given that doxorubicinol is too polar to allow partitioning back from

plasma to the cardiac muscle (23, 29). The more pronounced increase (50%) of measurable plasma aglycone is difficult to interpret in clinical terms. These metabolites are viewed at present as possibly implicated in the acute cardiac failure induced by anthracyclines, an event occurring rarely after one or two courses of anthracyclines (28). Assuming that the mechanisms of anthracycline- and trastuzumab-associated cardiotoxicity are different, the observed pharmacokinetic interactions may explain the higher incidence of asymptomatic and reversible LVEF decreases in cohort 1. Alternatively, the absence of even moderate effects on LVEF in cohort 2 indicates that trastuzumab therapy shortly after a brief course of anthracyclines is unlikely to affect cardiac function significantly.

The noncardiac side effects observed and their incidence were as expected whether trastuzumab was initiated with AT or after AT with paclitaxel alone (6, 7, 30, 31). Mild to moderate alopecia, paresthesia, and nausea were the most common treatment-related events. No patient had to be withdrawn from the trial because of toxicity, and the need for dose modification was limited. Together, these data indicate that this regimen is feasible for treating breast cancer regardless of when trastuzumab is initiated.

The ORR was high (87.5%) in both cohorts. Median TTP was longer in cohort 1 than cohort 2 (20.36 *versus* 12.76 months), although this difference is attributable to differences in the follow-up in the two cohorts. Both ORR and TTP were higher than in trials in which trastuzumab was combined with doxorubicin or paclitaxel individually (ORR ranging from 50 to 80% and TTP from 7.4 to 9 months; Refs. 8, 32, 33), although no conclusions can be drawn because of the small numbers of patients treated in this study, the inclusion of five patients with stage IIIB disease in each cohort, and the treatment of all stage IV patients first line. Patients in earlier trials all had stage IV disease, and some were treated second or third line (8, 32, 33).

In previous trials, patients with more strongly HER2-positive disease (IHC 3+ or fluorescence *in situ* hybridization positive) obtained the greatest benefit from trastuzumab-containing therapy (6, 34, 35). Interestingly, seven patients in cohort 1 had IHC 2+ disease, having been enrolled before full description of their differing sensitivities to trastuzumab. Thus, it might have been expected that individuals in cohort 1 would not respond to therapy as well as those in cohort 2. Patient numbers preclude any comparison based on HER2 status, but it is interesting that both patients in cohort 1 with stable disease as their best response had HER2 2+ breast cancer.

We also examined changes in HER2 ECD levels with therapy. All but one of the patients with elevated pretreatment ECD levels responded to treatment. Our findings suggest that high ECD levels do not predict resistance to treatment and are consistent with recent studies showing that patients with high ECD levels do not have enhanced resistance to trastuzumab and chemotherapy or chemotherapy alone (36, 37). Furthermore, because elevated ECD levels may be a marker for a subgroup of tumors with higher levels of HER2 cleavage and a more aggressive clinical course (38), the high level of response is encouraging. Finally, ECD levels were an early indicator of response to treatment in this trial and may be useful in monitoring the activity of this regimen. However, the utility of ECD levels in

predicting response to therapy requires investigation in larger randomized trials.

In conclusion, trastuzumab appears to be highly effective when given concomitantly with AT or with paclitaxel alone after completion of AT. Cardiotoxicity may be worse, but manageable with close cardiac monitoring, when trastuzumab is given concomitantly with AT. The findings of this study, together with the excellent antitumor activity of AT followed by CMF as neoadjuvant therapy for operable breast cancer (24), justified the recent initiation of a multicenter, randomized, controlled Phase III trial comparing AT followed by paclitaxel then by CMF with the same chemotherapy plus trastuzumab in women with HER2-positive locally advanced breast cancer.

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