A Prospective Randomized Pilot Study to Evaluate Predictors of Response in Serial Core Biopsies to Single Agent Neoadjuvant Doxorubicin or Paclitaxel for Patients with Locally Advanced Breast Cancer

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ABSTRACT

Introduction: Response to neoadjuvant chemotherapy for locally advanced breast cancer can be correlated with long-term outcomes. Surrogate end-point biomarkers may be used to assess response to the treatment. Most reported studies assessed the effects of combination chemotherapy. We assessed the feasibility of obtaining serial core breast biopsies, and correlated rates of apoptosis, proliferation, and expression of related proteins at baseline, during, and after neoadjuvant single agent chemotherapy for locally advanced breast cancer with response.

Experimental Design: Women with a histologically confirmed unresected T3 or T4 infiltrating carcinoma of the breast were eligible. The first 20 patients received three cycles of doxorubicin 90 mg/m2 followed by three cycles of paclitaxel 250 mg/m2, or the reverse. Nine women received four cycles of each (doxorubicin 60 mg/m2 and paclitaxel 175 mg/m2). Cycles were administered 14 days apart with filgastrin. End points included: (a) clinical and pathological response; (b) serial apoptotic [terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling] and proliferation (immunohistochemistry, IHC) rates; and (c) expression (IHC) of estrogen receptor, HER2, bcl2, and p53.

Results: From April 1997 to June 2001, 29 women were randomized. Twelve patients (42%) had a clinical complete response (cCR), and 16 (55%) had a clinical partial response. Five women (17%) had a pathological complete response, 7 (24%) had microscopic residual disease, and 17 (58%) had macroscopic residual disease. Higher baseline apoptosis and proliferation were associated with an improved pathological response (P = 0.006 and 0.003, respectively). Among 14 evaluable patients, apoptosis increased in women who had a cCR to the first agent but not in women without a cCR. Estrogen receptor-positive patients had a worse pathological response (P = 0.004).

Conclusions: The selected regimen is efficacious. It is feasible to obtain serial core biopsies that are informative for studies of apoptosis and IHC. This clinical design can serve as a model for combining standard chemotherapy and novel agents.

INTRODUCTION

Despite the widespread use of chemotherapy in cancer, the molecular signatures of the activity of individual agents on tumor cells during therapy are not well characterized. Although breast cancer mortality has declined in recent years, many women will not benefit from treatments available currently (1). Traditional end points to assess treatment efficacy, such as time to progression, disease-free survival, and overall survival require many months or years of follow up and large numbers of patients. Thus, elucidating surrogate molecular or cellular markers of efficacy of traditional agents may provide earlier insights into drug sensitivity and resistance.

Neoadjuvant or primary chemotherapy is generally administered to women with LABC.2 This treatment modality allows for accurate tumor measurements, assessment of response to therapy, and serial determinations of intratumoral characteristics (2, 3). In addition to enhancing the likelihood of breast preservation, response to primary chemotherapy can be correlated with long-term outcomes such as disease-free and overall survival (4–6).

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3 The abbreviations used are: LABC, locally advanced breast cancer; A, doxorubicin; T, paclitaxel; ER, estrogen receptor; cCR, clinical complete response; pCR, partial response; cSD, stable disease; pCR, pathological complete response; MiR, microscopic residual disease; MaR, macroscopic residual disease; TUNEL, terminal deoxynucleotidyl transferase (Tdt)-mediated nick end labeling; IHC, immunohistochemistry; C1D3, day 3 of cycle 1; C4D1, day 1 of cycle four.
Surrogate end point biomarkers might be used to rapidly predict response to neoadjuvant chemotherapy. Serial determinations of markers that represent downstream common events of biochemical and physiological pathways may be useful in predicting sensitivity or resistance to specific therapies. Insight into chemotherapy-induced biomarker changes might be used to develop and study new therapeutic agents, administered either alone or in combination with standard therapies.

Promising surrogate biomarkers of response to therapy include induction of apoptosis, reduced proliferation, and changes in markers related to the signal transduction pathways perturbed by the agent. An increase in rate of apoptosis was observed as early as 24 h after treatment in women who received primary combination chemotherapy for breast cancer (7). In patients with primary operable breast cancer who receive preoperative chemotherapy, improved response and survival were associated with a decline in proliferation rate (Ki67) after treatment (8, 9).

Previous reports of biomarkers in preoperative therapy have studied regimens of combination of several agents, including chemotherapy and hormone therapy (9). Doxorubicin (Adriamycin) is considered the most active agent in breast cancer (10). Recent studies have suggested that paclitaxel (Taxol®) is equally effective in metastatic breast carcinoma (11). Furthermore, paclitaxel is effective frequently in patients treated previously with doxorubicin, suggesting a relative lack of cross-resistance (12). Despite the widespread use of these agents, the molecular markers of response or resistance to either doxorubicin or paclitaxel have not been studied intensively in clinical human breast cancer. Single agent sequential treatment with dose-dense anthracyclines and taxanes has been extensively studied and is considered a promising approach (13, 14). We initiated a prospective pilot clinical trial to: (a) assess the feasibility of obtaining serial breast biopsies in patients receiving single agent sequential neoadjuvant doxorubicin followed by paclitaxel or paclitaxel followed by doxorubicin for LABC; (b) assess the feasibility of performing assays of apoptosis and of markers that predict response on the specimens obtained; (c) correlate the results of these assays with clinical and pathological response; and (d) compare the results of the assays in patients treated with single agent doxorubicin versus single agent paclitaxel.

**PATIENTS AND METHODS**

**Eligibility.** Women eligible for the study included those who were 18 years or older, who were not pregnant or lactating, with a histologically proven unresected clinical T3 or T4 infiltrating carcinoma of the breast. Women with prior hormonal or cytotoxic therapy, or those with a known severe comorbid medical or psychiatric conditions, were excluded. Eastern Cooperative Oncology Group performance status 0–2, a baseline left ventricular ejection fraction >50%, and adequate blood counts, and renal and liver functions were required.

**Clinical Trial Design.** After signing an informed consent approved by the Institutional Review Board at Georgetown University Medical Center, eligible participants underwent a baseline breast biopsy and were randomly assigned to three cycles of A 90 mg/m² followed by three cycles of T 250 mg/m², or the reverse sequence (A→T or T→A; Fig. 1). Cycles were administered 14 days apart, with granulocyte colony-stimulating factor support. The purpose of randomly assigning patients to the sequence was to allow for tumor sampling before and after treatment with either single agent doxorubicin or paclitaxel. To ensure equivalence between the two arms, the randomization was stratified by menopausal status (pre/perimenopausal or postmenopausal) and lesion stage (T3 or T4), and conducted in blocks of 4 patients as implemented in the RANLST module of the STPLAN software package (15). Midway into accrual to this trial, reports from several large prospective clinical trials suggested that higher doses of single agent chemotherapy were clearly more toxic and might not be more beneficial than standard doses (16–19). Therefore, for patients 21–29, the regimen was changed to four cycles of A at 60 mg/m² followed by four cycles of T at 175 mg/m², given 14 days apart, with granulocyte colony-stimulating factor support, or the reverse.

After the chemotherapy, patients underwent a modified radical mastectomy or a lumpectomy and axillary node dissection at the discretion of the treating breast surgeon. Postoperative chemotherapy was administered at the discretion of the treating medical oncologist. Breast or chest wall irradiation was administered at the conclusion of all of the chemotherapy. After irradiation, all of the women whose tumors were ER and/or progesterone receptor positive started tamoxifen therapy.

**Response Criteria.** Bidimensional tumor measurements were obtained before each cycle and 2 weeks after the last cycle of chemotherapy. Response to the regimen and to each agent was determined using Union Internationale Contra Cancrum criteria (20). CCR was defined as the disappearance of all of the measurable clinical disease. CPR was defined as a reduction of >50% in the product of the perpendicular dimensions of the

![Fig. 1](image-url)
Neoadjuvant Doxorubicin and Paclitaxel in Breast Cancer

defined as change in biomarkers with pCR, or with the presence of MiR, invasive carcinoma in the breast (6). We correlated baseline and disease by the study pathologist. pCR was defined as absence of specimen was carefully evaluated for the presence of residual procedure on completion of the regimen. The definitive surgical breast. All of the women underwent a definitive surgical pro-
cess for the purpose of this study for determination of potential invasive carcinoma throughout the specimen. We correlated baseline and change with response to the treatment in the breast. All of the women underwent a definitive surgical procedure on completion of the regimen. The definitive surgical specimen was carefully evaluated for the presence of residual disease by the study pathologist. pCR was defined as absence of invasive carcinoma in the breast (6). We correlated baseline and change in biomarkers with pCR, or with the presence of MiR, defined as < 1 cm, or MaR, defined as ≥ 1 cm or multiple foci of invasive carcinoma throughout the specimen.

Breast Biopsy. Serial core biopsies were obtained exclusively for the purpose of this study for determination of potential predictive surrogate markers of response. A core biopsy was obtained using Bard Monopty disposable biopsy instrument (Covington, GA). Two to four core biopsies were taken before starting chemotherapy (1–7 days before starting therapy), 24–48 h after cycle one (C1D3), and on C4D1 or sooner if a crossover between the agents had occurred. One to two core-biopsy specimens were fixed overnight in 10% buffered formalin and embedded in paraffin. Paraffin-embedded tissues were sectioned into 4 μm-thick sections and mounted onto slides. An additional core-biopsy specimen was rapidly frozen in liquid nitrogen and immediately placed in a −80°C freezer.

Apoptosis Assays. Apoptotic index was determined using the TUNEL method (TumorTACS In Situ Apoptosis Detection Kit; Trevigen, Inc., Gaithersburg, MD). Slides were deparaffinized, hydrated, and endogenous peroxidase was removed. Slides were incubated in labeling buffer for 5 min and then for 1 h at 37°C in a humid chamber with labeling buffer containing TdT, deoxynucleoside triphosphate mix, and Mn2+. Slides were then transferred into stop buffer for 5 min and washed in PBS. Streptavidin-horseradish peroxidase was applied onto each sample for 10 min. Slides were washed in PBS twice, placed in diaminobenzidine for 3 min, and counterstained with methyl green. Slides were dehydrated and mounted. Apoptosis index was calculated by counting and dividing the number of brown-stained nuclei by the total number of cells seen by light microscopy field at ×400 magnification by a blinded investigator (B. S.), and are expressed by percentage.

HIC. Markers for proliferation (Ki67), expression of ER, HER2/neu, bcl2, and p53 were evaluated by IHC. Slides were deparaffinized and hydrated. Antigen retrieval consisted of 10 mM sodium citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked with peroxide block (BioGenex, San Ramon, CA) and slides washed with PBS. Nonspecific antibody binding was blocked with normal goat serum (BioGenex) for 15 min at room temperature, and slides were washed with PBS. Separate slides were immunostained with commercially available antibodies (Table 1). Primary antibodies were diluted 1:20–1:50 in primary antibody diluent (BioGenex) and applied to the appropriate slide. After incubation at 37°C for 1–2 h, slides were washed in PBS, incubated with secondary antibody (Link; BioGenex) for 20 min at room temperature, washed in PBS, incubated with avidin-biotin complex (Label; BioGenex) for 20 min at room temperature, and again washed with PBS. Slides were stained with diamobenzidine, counterstained with hematoxylin and bluing solution (Optimax Wash Buffer; BioGenex), dehydrated, and mounted. IHC was scored for percentage of positive cells and/or relative intensity by a blinded investigator (B. S.) using light microscopy. The antibodies, manufacturers, and scoring system are listed in Table 1.

Statistical Methods. The primary end point, feasibility of serial biopsies, was not addressed statistically. Additional end points were addressed as follows. Patient baseline characteristics, the treatment regimen, and molecular markers were each assessed for an association with clinical or pathologic response using the Jonckheere-Terpstra test (21). Clinical and pathologic responses to the regimen were reported as the proportion of patients responding to therapy of the total number of patients enrolled on the study, after the intent-to-treat principle. For response to each specific agent, only those patients who were evaluable for response to that agent are included in the response rate. A patient was considered ineligible for response to the second agent if she achieved a cCR to the first agent or if she did not receive the second agent because of early withdrawal. All of the patients were considered eligible for response to the first agent.

Levels of apoptosis were compared by treatment response

### Table 1 Antibodies and procedures used for IHC

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>MAb&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Zymed, South San Francisco, CA</td>
<td>Nuclear staining. % positive</td>
</tr>
<tr>
<td>ER</td>
<td>MAb</td>
<td>Immunotech, Wesbrook, ME</td>
<td>Nuclear staining. ≥ 10% = positive</td>
</tr>
<tr>
<td>HER2 (erbB-2)</td>
<td>MAb</td>
<td>Novocastra, Vector Laboratories, Burlingame, CA</td>
<td>“DAKO system”</td>
</tr>
<tr>
<td>bcl-2</td>
<td>MAb</td>
<td>DAKO, Carpinteria, CA</td>
<td>Cytoplasmic staining.</td>
</tr>
<tr>
<td>p53</td>
<td>MAb (1801)</td>
<td>Novocastra, Vector Laboratories, Burlingame, CA</td>
<td>Nuclear staining. ≥ 10% = positive</td>
</tr>
</tbody>
</table>

<sup>a</sup> MAb, monoclonal antibody.
level using a one-way ANOVA to assess whether apoptosis after the first agent was associated with pathologic response (22). Survival and follow-up estimates were calculated using the methods of Kaplan and Meier (23).

RESULTS

Clinical Data. From April 1997 through June 2001, 29 women enrolled in the trial and completed the regimen. The patient characteristics and response to treatment are summarized in Tables 2, A and B. Twelve women (42%) had a cCR. Of those, 7 patients had a cCR to the first drug (A→T = 2; T→A = 5) and 5 to the second drug (A→T = 3; T→A = 2). Four cCR were observed while administering A and 8 cCR while administering T. Sixteen patients (55%) had a cPR to the regimen (overall response rate 97%). One woman had a cSD. Clinical response was similar in patients who received A→T or T→A (Table 2B).

Five women had a pCR in the breast (17%). Of these 5 women, 3 had a pCR in the breast and the lymph nodes (10%). Seven women (24%; A→T = 2; T→A = 5) had MiR in the breast (<1 cm). Seventeen patients (59%; A→T = 10; T→A = 7) had MaR in the breast (≥1 cm or multiple foci). Pathological response was similar in patients who received A→T or T→A (Table 2B).

Of the 16 women with an initial T3 lesion, 7 (44%) underwent successful breast conserving surgery. After the surgical procedure, 22 of 24 women with a stage III disease received additional chemotherapy, usually single agent cyclophosphamide. Six of these women also received high-dose chemotherapy and peripheral stem cell rescue. Two women relapsed soon after their surgery and were treated for metastatic disease. These 2 women did not receive irradiation. One additional woman relapsed while receiving cyclophosphamide and did not receive breast irradiation. A fourth woman did not receive chest wall irradiation because of a previous irradiation to the same breast. 22 of 24 women with a stage III disease received additional chemotherapy, usually single agent cyclophosphamide.

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disease recurrence, and all 5 of the (100%) women with stage IV disease suffered disease progression. Nine (38%) women with stage III and 2 (40%) women with stage IV breast cancer died of their disease. One additional patient with a stage IIIA disease died without clinical evidence of recurrent disease.

Grade 3 and 4 toxicities were common for the first 20 patients and were mostly hematological. Of the first 20 women, 35% and 30% suffered grade 3/4 leukopenia and neutropenia, respectively, and 25% suffered grade 3 neutropenic fevers. Lymphopenia was reported in 3 women (15%). Of the first 20 patients, 15 did not receive erythropoietin, and 7 (44%) required blood transfusions (median 2 units of packed RBCs, after three to six cycles of chemotherapy), whereas 5 patients received erythropoietin, and none required blood transfusions. Grade 3/4 nonhematological toxicities in the first 20 patients included gastrointestinal (30%), fatigue (15%), bone pain/arthralgias (15%), hand foot syndrome (10%), and peripheral neuropathy (5%).

Of the 9 women who received the less intense regimen, none suffered grade 3/4 leukopenia, neutropenia, nor required erythropoietin or blood transfusions. Five women (56%) suffered lymphopenia. One woman suffered a grade 3 motor neuropathy from paclitaxel and was removed from the study. Other chemotherapy-related grade 3/4 toxicities included infection (22%) and arthralgias (11%). One of the women who suffered infection was taken off study.

**Correlative Tissues.** Biopsies and tissues available for analysis are summarized in Table 3. All 29 of the women underwent a baseline breast biopsy. Twenty (69%) of the baseline samples that were obtained specifically for the study contained sufficient cancer cells for marker analysis. Whenever sufficient tissue was not available at the time of the study-specific baseline biopsy, the diagnostic paraffin block was retrieved, and sections were cut and used for baseline marker analysis. Because of improper fixation in the beginning of the trial, several samples were not evaluable for baseline apoptosis determination (Table 3). After we observed that ethanol fixation produced false-positive TUNEL staining, all of the tissues were fixed in formalin. Therefore, we were able to perform studies of apoptosis and proliferation on 26 and 27 patients at baseline, respectively. Twenty-five (86%) women had a biopsy on C1D3, of which only 17 contained infiltrating carcinoma sufficient for IHC staining, and only 14 samples were evaluable for apoptosis determination. Thus, 14 women had paired samples before and after the first cycle of chemotherapy evaluable for apoptosis.

Initially, a biopsy was attempted on C4D1. Of the first 14 women, 11 biopsies were attempted at the C4D1 time point. Whereas 7 samples contained cancer cells, only 2 contained a sufficient number of malignant cells for proper marker analysis. Thus, subsequent biopsies were obtained on C4D1 only when a distinct mass was palpated. Finally, representative slides from the definitive surgical specimen were evaluated by the study pathologist (B. S.), and only 16 specimens (55%) contained invasive carcinoma in the breast sufficient for marker analysis (Table 3).

**Apoptosis and Proliferation.** Baseline apoptosis and proliferation were evaluated in 26 and 27 samples respectively (Figs. 2, A and B). Mean baseline TUNEL and ki67 staining...
scores for all of the study participants were 0.34% and 33%, respectively. For patients who achieved a pCR, mean baseline values of apoptosis and proliferation were 0.52% and 46%, respectively. Mean apoptotic or proliferative indices were 0.7% and 60% for patients who had MiR in the breast, and 0.16% and 24% for those with MaR, respectively ($P = 0.003$ and 0.006 for apoptosis and proliferation, respectively).

Apoptotic index on C1D3 was compared with baseline index in 14 evaluable patients (Fig. 3). Because the second sample was taken after the first cycle of chemotherapy, correlations were made only with the clinical response to the first agent and not to the second agent. The 3 women who had a cCR to the first agent had an increase in apoptosis (4-fold, 20-fold, and 1 was not evaluable because of high degree of necrosis). In contrast, in 11 women with a partial or no response to the first agent, we did not observe a significant increase in apoptosis. Mean baseline apoptosis for the 11 patients who did not have a cCR to the first agent was 0.34% (range, 0–1%), and at C1D3 0.45% (range, 0.1–1%). Although the sample size is small, these results suggest that an increase in apoptosis may be observed in women who had a cCR to the first agent, whereas no significant change may be detected in women who had cPR or cSD to that agent.

**Predictive Markers of Response to Chemotherapy.**

We correlated pathological response to A→T or T→A (pCR, MiR, or MaR in the breast) with baseline status of ER, HER2, bcl2, and p53. MiR was seen in 14% of women whose tumors expressed ER compared with 33% pCR and 33% MiR in women whose tumors were ER poor ($P = 0.004$; Fig. 4A). Overexpression of HER2 was associated with 30% pCR and 30% MiR compared with 11% pCR and 21% MiR in HER2-negative tumors ($P = 0.169$; Fig. 4B). No statistically significant difference in pathological response was observed based on bcl2 expression ($P = 0.879$; Fig. 4C) or p53 expression ($P = 0.463$; Fig. 4D).

We then evaluated pretreatment characteristics and response to specific agent A or T. To determine response to each agent, we calculated clinical response (cCR versus cPR/cSD) to the first agent and to the second agent (Fig. 1. Clinical Response 1 and 2, respectively). Because the patients received both single agents sequentially before undergoing surgery, correlation was made between baseline tumor marker result and clinical response rather than pathological response. Furthermore, women who had a cCR to the first chemotherapy agent had no measurable disease in the breast before starting the second agent and were not evaluable for response to the second agent. For example, if a woman in regimen A→T had a cCR to A, we concluded that the cancer was sensitive to A but could not assess the sensitivity to T. In contrast, if another woman who received A→T had cPR or cSD to A, we evaluated the clinical response to A (tumor measurement before and after A) and to T (tumor measurement before and after T). Seven women had cCR to the first agent (2 to A and 5 to T) and, thus, were not evaluable for response to the second agent. In addition, 2 women were taken off study after receiving only one cycle of the second agent (A→T = 1 and T→A = 1). Thus, only 23 women were evaluable for clinical response to A, and 26 were evaluable for clinical response to T (Table 4).

In ER-positive patients, the overall response to either A or T was poorer than for ER-negative patients (ER-positive: 8% cCR to A and 15% cCR to T; ER-negative: 36% cCR to A and 42% cCR to T; Fig. 5A). However, women whose tumors overexpressed the HER2 oncogene were more likely to achieve a cCR to A than to T (Fig. 5B; HER2-positive 56% cCR and HER2-negative 0% cCR to A). Furthermore, HER2-positive patients were slightly more likely to have cCR to T (HER2-positive 33% cCR and HER2-negative 26% cCR to T), but this difference was not as dramatic as that observed with A. Of note, 3 of the 6 women with tumors overexpressing HER2 who were on regimen A→T had a cCR to A and were, therefore, not evaluable for response to T. Another woman received only one of four scheduled cycles of T and was, thus, not evaluable for response to T. We did not observe a difference in response to individual agent A or T based on bcl2 or p53 expression (Fig. 5, C and D, respectively). Finally, we did not observe significant changes in expression of ER, HER2, bcl2, or p53 during or after the chemotherapy (data not shown).

**DISCUSSION**

In this pilot prospective trial, we studied baseline and changes in surrogate end point biomarkers after single agent sequential administration of doxorubicin or paclitaxel as neoadjuvant chemotherapy for LABC. High rates of apoptosis and proliferation at baseline were associated with improved pathological response to both regimens (A→T or T→A). Increased rate of apoptosis 24–48 h after treatment with doxorubicin or paclitaxel was associated with improved clinical response.

Our results are similar to other reports in the literature. As reported previously, we observed that adequate training and experience are required to obtain adequate tissue for analysis (24). Nonetheless, our data are consistent with reports that early changes in apoptosis correlate with subsequent response to the regimen (7). In addition, prospective evaluation of response to single agent therapy may permit us to evaluate molecular signatures of sensitivity or resistance to individual agents. On the basis of these characteristics, several hypotheses can be generated.

One hypothesis is that breast carcinomas with very low baseline apoptosis may have a poor response to chemotherapy.
The sample size in this study was too small to conduct a multivariate analysis, but it appeared that tumors with low apoptotic ratios were ER-positive. Other investigators have assessed the effects of chemoendocrine therapy on proliferation and did not observe a difference in response rates for ER-rich or ER-poor tumors (8, 25). Our results are consistent with retrospective evaluations that suggested that hormone receptor-negative tumors are more chemosensitive than those that lack

![Fig. 4](image-url)

**Table 4** Evaluable patients for correlation of baseline markers and response to chemotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>n (%)</th>
<th>Clinical response</th>
<th>Pathological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients in A→T arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluable for response to A</td>
<td>15 (52)</td>
<td>14 (93)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>cCR to A</td>
<td>15 (52)</td>
<td>13 (87)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Stopped T early to do AE</td>
<td>2 (10)</td>
<td>3 (10)</td>
<td>2 (67)</td>
</tr>
<tr>
<td>Evaluable for response to T</td>
<td>12 (38)</td>
<td>9 (75)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>cCR to T</td>
<td>12 (38)</td>
<td>9 (75)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Patients in T→A arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluable for response to T</td>
<td>14 (48)</td>
<td>14 (100)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>cCR to T</td>
<td>14 (48)</td>
<td>13 (93)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Stopped A early to do AE</td>
<td>5 (17)</td>
<td>5 (17)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Evaluable for response to A</td>
<td>8 (28)</td>
<td>8 (28)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>cCR to A</td>
<td>8 (28)</td>
<td>8 (28)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Patients in A→T or T→A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluable for response to A</td>
<td>23 (79)</td>
<td>23 (79)</td>
<td>12 (41)</td>
</tr>
<tr>
<td>cCR to A</td>
<td>23 (79)</td>
<td>19 (83)</td>
<td>12 (41)</td>
</tr>
<tr>
<td>Evaluable for response to T</td>
<td>4 (17)</td>
<td>4 (17)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>cCR to T</td>
<td>4 (17)</td>
<td>4 (17)</td>
<td>4 (100)</td>
</tr>
</tbody>
</table>

* A patient is considered to have a clinical response if she had either cCR or cPR.

* A patient is considered to have a pathological response if she had either pCR or MiR.

* AE, adverse event.
hormone receptors in both the metastatic and neoadjuvant settings (6, 26, 27). It is now clear that patients with ER-positive tumors benefit substantially from endocrine manipulations (28). Such observations have led to hypotheses that chemotherapy may add little benefit to adequate hormone therapy in receptor-rich patients, although this hypothesis requires confirmation in prospective randomized clinical trials.

A second hypothesis that can be generated is that higher baseline apoptotic rates are associated with better response to chemotherapy. Therefore, if available, pretreatment with agents that specifically induce apoptosis might result in high response to chemotherapy. We suggest that this trial design is a good clinical model to test this theory.

These data also suggest that it may be possible to determine as early as 24–48 h after administration of chemotherapy whether a woman is likely to respond to a specific agent or not. Such information might help to make early decisions regarding a change in treatment. Moreover, the single agent nature of this trial design permits assessment of changes in surrogate markers (such as increased apoptosis) in a more straightforward fashion than if the chemotherapy is given in combination. Larger studies are required to assess whether anthracyclines and taxanes induce different changes in apoptosis or proliferation. Such results may provide biological insights into the mechanisms of action of both standard and novel antineoplastic treatments.

The novel approach in this study can also answer questions regarding the role of other markers and response to individual therapies. For example, it has been suggested that overexpression or amplification of HER2 may be associated with an improved response to doxorubicin and possibly paclitaxel (29, 30). Our data are also consistent with this hypothesis.

Other markers that are closely related to cell cycle and cell death include bcl2 and p53. Although we did not observe a significant correlation between expression of bcl2 and response to the chemotherapy, we have not evaluated posttranslational modification of the protein nor ratio with other bcl2 family members. It is also possible that other methods of detecting an alteration in a gene or a gene product may be more sensitive to predict for response to specific therapy. One study suggested that wild-type p53 was associated with an improved response to anthracyclins, whereas a mutated p53 was associated with an improved response to paclitaxel (31).

In summary, we present a clinical design incorporating single agent sequential chemotherapy in LABC that can be used in future investigation not only to correlate surrogate end point biomarkers with response to single agent therapy. The model can also be used to incorporate novel agents with standard treatments. Changes in apoptosis and proliferation can be used to determine the efficacy of the combination.

![Fig. 5](image_url)
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A Prospective Randomized Pilot Study to Evaluate Predictors of Response in Serial Core Biopsies to Single Agent Neoadjuvant Doxorubicin or Paclitaxel for Patients with Locally Advanced Breast Cancer

Vered Stearns, Baljit Singh, Theodore Tsangaris, et al.


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