Neoadjuvant Docetaxel and Capecitabine and the Use of Thymidine Phosphorylase as a Predictive Biomarker in Breast Cancer

Rachel M. Layman,[1] Dafydd G. Thomas,[2] Kent A. Griffith,[3] Jeffrey B. Smerage,[1] Mark A. Helvie,[4] Marilyn A. Roubidoux,[4] Kathleen M. Diehl,[5] Lisa A. Newman,[5] Michael S. Sabel,[5] James A. Hayman,[6] Lori J. Pierce,[6] Daniel F. Hayes,[1] and Anne F. Schott[1]

Abstract

Purpose: Thymidine phosphorylase (TP) induction by docetaxel is a proposed mechanism for the observed preclinical synergy of docetaxel and capecitabine (DC). We evaluated whether TP protein expression is increased by docetaxel and correlates with pathologic complete response (pCR) in breast cancer patients.

Experimental Design: Women with stage II to III breast cancer were given four cycles of neoadjuvant docetaxel 36 mg/m^2 i.v. over 30 min on days 1, 8, and 15 and capecitabine 2,000 mg/d, in two divided doses, on days 5 to 21 of a 28-day cycle. Radiology-directed biopsies of the breast tumors were done at baseline and 5 days after the first dose of docetaxel to evaluate TP expression. Following DC therapy, patients had core breast biopsies, and if residual disease was present, received four cycles of standard dose-dense doxorubin and cyclophosphamide (AC).

Results: The pCR rate was 26.9% (95% confidence interval, 11.6-47.8). Up-regulation of TP expression was not observed by either quantitative immunofluorescence (QIF) or immunohistochemistry. Radiology-directed core biopsy after neoadjuvant chemotherapy accurately predicted pathologic response in 88% (95% confidence interval, 69.8-97.6) of the cases. Neither level of TP expression nor TP up-regulation correlated with pCR. Significant toxicity resulted in therapy discontinuation in 3 of 26 patients.

Conclusions: DC chemotherapy exhibited a similar pCR rate compared with standard taxane regimens, with increased toxicity. TP expression was not up-regulated after docetaxel and did not correlate with therapeutic response. Core breast biopsy after neoadjuvant chemotherapy accurately predicted pathologic response.

Preoperative, or "neoadjuvant" chemotherapy for breast cancer results in similar overall survival with higher breast preservation rates compared with postoperative chemotherapy (1). Neoadjuvant therapy also allows the clinician to observe the response to treatment and provides an opportunity for biological evaluation of the primary tumor before and after chemotherapy (2). Observation of response to treatment may allow therapy to be tailored to individual patients (3), which has potential to improve response and survival rates for some patients, and spare others toxicity from ineffective therapy. The ability to tailor chemotherapy based on pretreatment predictors or early markers of response to preoperative therapy would advance breast cancer treatment.

Standard taxane and anthracycline chemotherapy for high-risk breast cancer improves survival (4–6). However, approved drug combinations, such as doxorubicin, cyclophosphamide, and docetaxel, have only additive effects in preclinical models, rather than the synergy that has been proposed for other regimens, such as docetaxel and capecitabine (DC; refs. 7, 8). Capecitabine is an orally bioavailable prodrug to 5-fluorouracil, which is preferentially converted in tumors through a process requiring the enzyme thymidine phosphorylase (TP; ref. 9). Docetaxel has been shown to increase the level of TP expression in cancer cell lines (7), possibly accounting for the synergistic inhibition of tumor growth by DC seen in models. Clinical trials have shown DC to be more effective in metastatic breast cancer than docetaxel alone (10, 11), supporting the further exploration of this drug combination in neoadjuvant and adjuvant therapy.

In colorectal cancer, it has been shown that tumor expression of the drug-metabolizing enzyme dipyridamole dehydrogenase

Authors' Affiliations: Departments of [1]Internal Medicine, [2]Pathology, [3]Biostatistics, [4]Radiology, [5]Surgery, and [6]Radiation Oncology, University of Michigan, Ann Arbor, Michigan

Received 2/5/07; revised 4/3/07; accepted 5/10/07.

Grant support: Fashion Footwear Association of New York/QVC Presents Shoes on Sale, Sanofi-Aventis Pharmaceuticals, Roche Pharmaceuticals, and the NIH through the University of Michigan's Cancer Center Support Grant 5 P30 CA46592. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: This research was presented at the 28th Annual San Antonio Breast Cancer Symposium, December 8-11, 2005.

Requests for reprints: Rachel M. Layman, Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan, C354 Med Inn Building, Box 0848, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0848. Phone: 734-615-4762; Fax: 734-647-8792; E-mail: rlayman@umich.edu.

doi:10.1158/1078-0432.CCR-07-0288

can predict the effectiveness of fluorouracil treatment (12). Similarly, TP is expressed at high levels in over 50% of breast cancer specimens (13–16), and elevated levels of TP have been associated with improved efficacy of capecitabine (17–19). Reported here are the results of a pilot study evaluating neoadjuvant DC for high-risk breast cancer, designed to evaluate the safety and efficacy of this treatment, and whether the intensity of TP expression in tumors at baseline or after docetaxel correlates with therapeutic response.

Materials and Methods

The study protocol was reviewed and approved by the University of Michigan Medical School Institutional Review Board. Each study participant signed an approved informed consent document before any study-specific procedures.

Eligibility criteria. Women with stage II to III breast cancer with measurable disease amenable to biopsy were enrolled. Histologic diagnosis was made by core needle or incisional biopsy and estrogen, progesterone, and HER-2 receptor status were determined by immunohistochemistry. Patients had an Eastern Cooperative Oncology Group performance status of 0 to 1 and normal organ function. Patients were excluded if they had a warfarin requirement, existing coagulopathy, significant cardiac disease, systemic malignancy within 1 year, major surgery within 4 weeks of treatment, or contraindications to capecitabine or docetaxel. Pregnant and nursing patients were ineligible. Patients could have no prior chemotherapy or radiation therapy for their current cancer, but may have received ≤4 weeks of hormonal therapy, which was discontinued during chemotherapy.

Pretreatment evaluation. Before chemotherapy, patients had staging of the axillary lymph nodes by combined ultrasound and fine needle aspiration of clinically suspicious lymph nodes, or with sentinel lymph node biopsy if no suspicious nodes were present, and placement of a metal clip at the tumor site. Baseline imaging with standard diagnostic mammography of the involved breast and three-dimensional ultrasound of the breast tumor on a GE Logiq 700 scanner was done. Research core needle biopsies for TP analysis were obtained by skilled providers using Tru-Cut (Baxter) disposable core-cutting needles, in most cases using radiologic guidance.

Treatment plan. Patients received four cycles of neoadjuvant DC. Docetaxel (Taxotere; Sanofi-Aventis Pharmaceuticals) 36 mg/m^2 i.v. was given over 30 min on days 1, 8, and 15 of each 28-day cycle. Fixed-dose capecitabine (Xeloda; Roche Laboratories) 2,000 mg/d, in two divided doses, regardless of patient body surface area (BSA), was given on days 5 to 21. Premedication with dexamethasone before docetaxel and vitamin B6 hand-foot syndrome prophylaxis was recommended. Growth factor support was permitted. In the case of excess toxicity, dose reduction guidelines were followed.

On day 5 of the first cycle of DC (after docetaxel and before beginning capecitabine), patients had repeat radiology-directed core biopsies for TP assessment.

Patients with progressive disease or excessive toxicity during DC had early discontinuation of DC and were offered salvage therapy. Responding and stable patients, after four cycles of DC, underwent radiology-directed core needle biopsies of their tumors. Patients with residual disease on core needle biopsy received additional chemotherapy, and those with apparent complete pathologic response (pCR) proceeded to definitive surgery.

Patients without pCR in both the breast and lymph nodes after four cycles of DC (as identified by core biopsy or definitive surgery) received four cycles of standard dose-dense doxorubicin (Adriamycin; Pharmacia) and cyclophosphamide (Cytoxan; Bristol-Myers Squibb; AC). Doxorubicin 60 mg/m^2 i.v.p. and cyclophosphamide 600 mg/m^2 i.v. were given on day 1 of each 14-day cycle. Filgrastim (Neupogen; Amgen) 300 μg s.c. was given on days 3 to 10 of the first three cycles of AC.

Patients with confirmed pCR to DC alone at definitive surgery received no further chemotherapy. Patients who received DC/AC before definitive surgery received no additional cytotoxic therapy after surgery (Fig. 1). Radiation therapy and hormonal therapy were administered to all patients, as clinically indicated.

TP assessment. Paraffin-embedded, methanol-fixed research biopsies were assessed for TP with quantitative immunofluorescence (QIF) and immunohistochemistry. Tissue sections were designated "tumor" versus "stroma" by direct inspection, sectioned, deparaffinized, and stained with immunofluorescence antibody for TP and nucleic acid.

After dewaxing, sections were microwave-preheated in citric acid buffer, permeabilized with 0.1% saponin for 10 min, and treated with three washes of 0.02 mol/L glycine and 0.1% paraphenyldiamine in TBS-Tween 20. Blocking solution (5% goat normal serum and 1% fetal

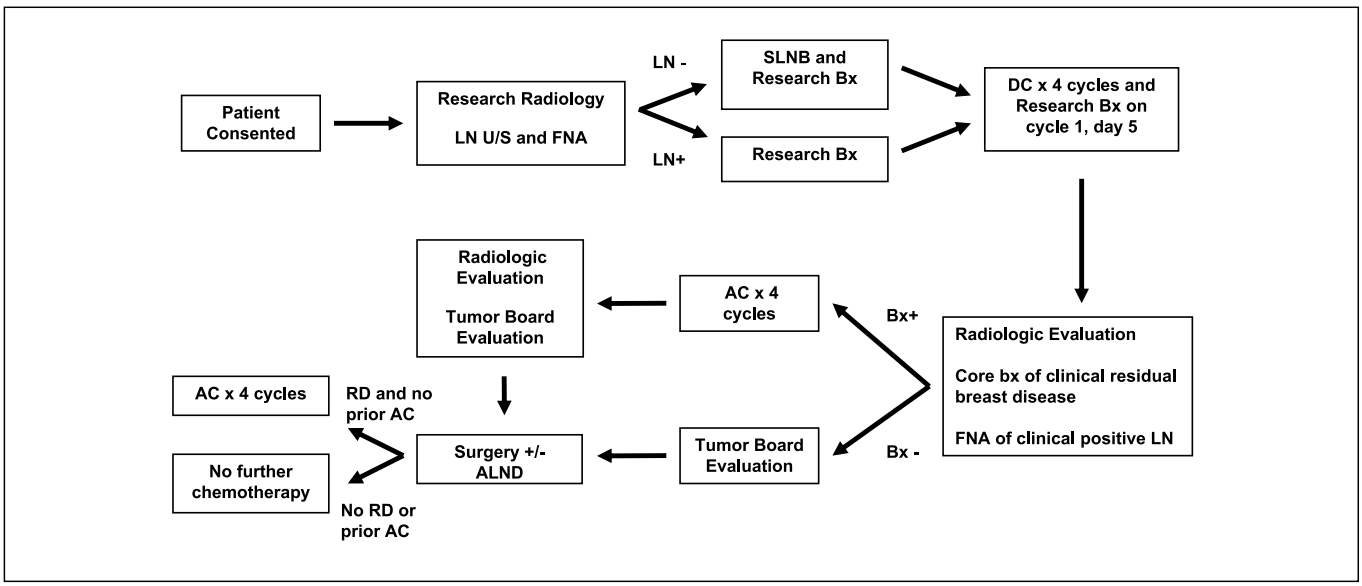

Fig. 1. Schema of clinical trial study design. LN, lymph node; U/S, ultrasound; FNA, fine needle aspiration; SLNB, sentinel lymph node biopsy; Bx, biopsy; ALND, axillary lymph node dissection; RD, residual disease.

bovine serum in TBS-Tween 20) was administered for 30 min before exposure to the primary TP antibody (mouse monoclonal antibody; Neomarkers) at 1:200 dilution (1 μg/mL) for 1 h at room temperature. Sections were exposed to the labeled secondary antibody (AF568 goat anti-mouse, Molecular Probes) at 1:250 dilution for 30 min. Cell nuclei were stained with Syto 16 (Molecular Probes; 1:4,000) for 30 min. The sections were mounted in antifading medium and kept in the dark at 4°C until examined. Labeled sections were initially excited at λ = 488 nm and the fluorescing nuclei images acquired at ×20 magnification by digital micrograph and UltraViewTM imaging software (Perkin-Elmer). Consequently, sections were excited at λ = 568 nm acquiring the fluorescing TP images. Computer-generated composite images of nuclei and antibody allowed visualization of the cellular distribution.

Tumor-rich biopsy regions were selected and images were partitioned into a grid of squares. Nuclear density and antigen-antibody complex pixel intensity were estimated within each square area. A weighted average of antibody intensity was computed for each tissue section weighted by normalized nucleic intensity.

Immunohistochemical staining was done on the DAKO Autostainer (DAKO) using DAKO LSAB+ and diaminobenzidine as the chromogen. Deparaffinized sections of methanol-fixed tissue at 5-μm thickness were labeled with an antibody to thymidylate phosphorylase (mouse monoclonal antibody, 1:200, Neomarkers, LabVision), after microwave citric acid epitope retrieval. Appropriate negative (no primary antibody) and positive controls (breast carcinoma) were stained in parallel with each set of tumors studied. The immunoreactivity was scored by a three-tier [negative, low-positive (1+) and high-positive (2+)] modification of the normal grading scheme previously described by Wang et al. (20).

Design and end point definitions. The primary efficacy end point was pathologic complete response (pCR) rate. Patients could have pCR to DC alone, or to DC followed by AC. For the former, there could be no residual invasive cancer in the lymph nodes, definitive surgery specimens, or directed tumor bed core biopsies, but ductal carcinoma *in situ* only would still be classified as pCR. Pathologic noncomplete response is defined as not meeting pCR criteria.

This trial accrued a planned 26 patients. Stopping rules for the trial were constructed starting with the 10th patient with continued sequential assessment until the final patient. Early termination was planned if there was 99% confidence that the experimental treatment was not more efficacious than historical data from the NSABP B-18 treatment, which reported a pCR rate of 13.7% after four cycles of AC (1). An introductory cohort of 10 patients were accrued and treated before the implementation of the stopping rules, calculated using the method of Thall and Simon (21).

The primary correlative end point was change in quantitative TP expression from baseline to day 5 biopsies. Correlative end point data was hypothesis generating and this trial was not powered, *a priori*, to test the correlative hypotheses.

TP expression was analyzed for a trend in increasing values between baseline and day 5 values. The signed-rank test of Wilcoxon was used to characterize, nonparametrically, whether the median difference was statistically different from zero. Baseline TP expression levels, TP at day 5, and changes over time were compared between patients with and without a pCR using the Wilcoxon rank-sum test statistic. Given the small numbers, the threshold of detecting a significant association was increased to the 10% significance level to guide the interpretation of a potential trend of association between TP overexpression and pathologic response.

Results

Patients. Twenty-seven patients consented to the study. Because one patient was diagnosed with metastatic disease during the screening period, 26 patients with stage II to III breast cancer were enrolled between February 2004 and April 2005. The majority of patients (57.7%) had estrogen receptor–negative/progesterone receptor–negative breast cancers (Table 1).

Table 1. Baseline patient characteristics (N = 26)

	n (%)
Age, y	
Median (range)	49 (29–67)
Size of tumor (cm; clinical)	
≤2.0	2 (7.7)
2.1-5.0	18 (69.2)
≥5.1	6 (23.1)
Prechemotherapy lymph node status (includes SLNB)	
Negative	6 (23.1)
Positive	20 (76.9)
Prechemotherapy stage (includes SLNB)	
I	0 (0)
II	19 (73.1)
III	7 (26.9)
Hormone receptor status	
ER+/PgR+	7 (26.9)
ER-/PgR-	15 (57.7)
ER+/PgR-	2 (7.7)
ER-/PgR+	2 (7.7)
Her-2/neu Status (positive required 3+ by IHC or FISH+)	
Negative	20 (76.9)
Positive	6 (23.1)

Abbreviations: SLNB, sentinel lymph node biopsy; ER, estrogen receptor; PgR, progesterone receptor; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Twenty-one patients received four cycles of DC and five did not complete DC–one patient had progressive disease, one had poor response, and three had discontinuation due to toxicity. All patients who did not attain pCR to DC received four cycles of AC. One patient who did not complete four cycles of DC due to toxicity subsequently received six cycles of AC. All patients were included in the intent-to-treat analysis.

Response. Of the 26 patients, two had pCR after four cycles of DC alone and five more attained pCR with combined DC and AC chemotherapy. Therefore, 26.9% (95% confidence interval, 11.6-47.8) of the participants achieved pCR, consistent with historical data of anthracycline- and taxane-based therapy (22). There was a trend of increased response rate in patients with estrogen receptor–negative/progesterone receptor–negative/Her-2–negative breast cancers with 41.7% pCR (95% confidence interval, 15.2-72.3) versus 14.3% (95% confidence interval, 1.8-42.8) for all others, which was not statistically significant (Fisher's exact P = 0.190).

Radiology-directed core biopsy after neoadjuvant DC chemotherapy accurately predicted pathologic response in 88% (95% confidence interval, 69.8-97.6) of the cases. Three patients with no evidence of residual disease on interim biopsy after DC had minimal residual invasive disease on definitive surgery and received four cycles of adjuvant AC.

Toxicity. Three patients discontinued DC secondary to toxicity. One patient developed hand-foot syndrome, diarrhea, mucositis, and rash. The second patient developed persistently mildly elevated transaminases and hyperbilirubinemia. A third patient developed a grade 3 infusion reaction during her first

Table 2. Adverse events by grade

Toxicity	No. patients with toxicity by grade (%)				
	Grade 1	Grade 2	Grade 3	Grade 4	Total (*N* = 26)
Hematologic					
Anemia	17 (65)	6 (23)	0	0	23 (88)
Leukopenia	3 (12)	11 (42)	4 (15)	0	18 (69)
Neutropenia	0	7 (27)	3 (12)	0	10 (38)
Thrombocytopenia	1 (4)	0	0	0	1 (4)
Nonhematologic					
Fatigue	21 (81)	2 (8)	0	0	23 (88)
Hyperglycemia	10 (38)	13 (50)	0	0	23 (88)
Hand-foot syndrome	4 (15)	12 (46)	5 (19)	0	21 (81)
Excessive tearing	13 (50)	6 (23)	1 (4)	0	20 (77)
Nausea/vomiting	14 (54)	6 (23)	0	0	20 (77)
Abnormal LFTs	14 (54)	3 (12)	0	0	17 (65)
Diarrhea	12 (46)	3 (12)	1 (4)	0	16 (62)
Nail changes	2 (8)	14 (54)	0	0	16 (62)
Alopecia	9 (35)	6 (23)	0	0	15 (58)
Myalgia/arthralgia	12 (46)	2 (8)	0	0	14 (54)
Dyspepsia	6 (23)	7 (27)	0	0	13 (50)
Mucositis	5 (19)	8 (31)	0	0	13 (50)
Infection	0	8 (31)	4 (15)	0	12 (46)
Taste change	10 (38)	2 (8)	0	0	12 (46)
Headache	10 (38)	0	0	0	10 (38)
Neuropathy	7 (27)	2 (8)	0	0	9 (35)
Chest pain	3 (12)	3 (12)	0	0	6 (23)
Cytokine release syndrome	0	2 (8)	1 (4)	0	3 (12)
Nephrolithiasis	0	0	1 (4)	0	1 (4)

NOTE: Highest grade of each adverse event per patient is shown.
Abbreviation: LFT, liver function tests.

docetaxel dose and never started capecitabine. An additional patient had capecitabine discontinued from the DC regimen during the first cycle due to angina requiring hospitalization. All acute toxicities resolved upon discontinuation of therapy.

Grade 3 treatment-related toxicities included hand-foot syndrome, leukopenia, diarrhea, cytokine release syndrome, angina, and infection. Two patients developed postprocedure breast cellulitis, one patient had a facial abscess, and another developed pyelonephritis. Additionally, one patient had persistent excessive eye tearing finally requiring surgical intervention ~1 year after the completion of chemotherapy. No grade 4 toxicities were observed. The most common grade 1 to 2 toxicities are shown (Table 2).

Thymidine phosphorylase. A representative specimen stained for TP by QIF is shown (Fig. 2).

TP was successfully assayed in pretreatment core biopsies for 26 patients and in day 5 biopsies for 24 patients. TP expression up-regulation was not observed as measured by either QIF (Fig. 3) or immunohistochemistry (Fig. 4). Neither baseline level of TP expression nor TP expression after docetaxel was associated with pCR.

Discussion

This study revealed a pCR rate of 26.9% after neoadjuvant chemotherapy with four cycles of DC followed by four cycles of dose-dense AC in patients with stage II to III breast cancer, consistent with historical data of similar regimens without the addition of capecitabine. For example, the NSABP B-27 study revealed a 26.1% pCR rate after four cycles each of neoadjuvant AC and docetaxel with 10.9% of patients in the study discontinuing docetaxel because of adverse events (22). In the present study, 15% of the patients required discontinuation of DC entirely or the capecitabine component due to toxicity. There were high rates of grade 1 and 2 toxicities, such as nail changes and excessive tearing, which were bothersome to the

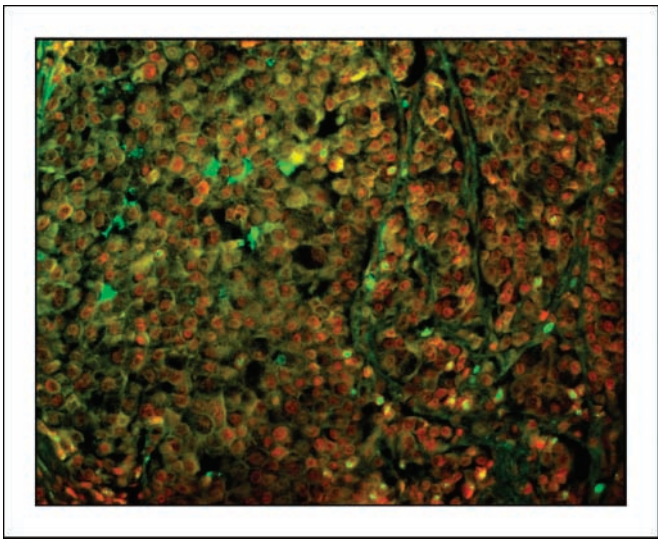

Fig. 2. QIF staining for TP. A representative specimen stained for TP expression by QIF. Images for the antigen-antibody complex labeled with Alexa-Fluor 568 (*red*) and nuclear density (*green*) were separately acquired and combined using the UltraView Software program. Original magnification, ×200.

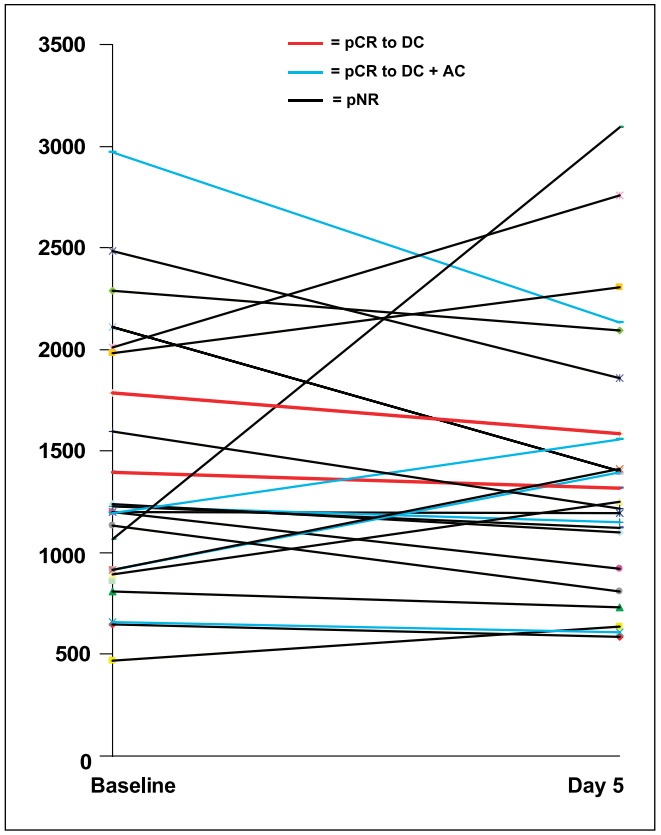

Fig. 3. TP measurement by QIF before and after docetaxel. TP expression is plotted at baseline and 5 d after the first dose of docetaxel stratified by pathologic response, demonstrating lack of correlation. pNR, pathologic noncomplete response.

patients. The results suggest that neoadjuvant DC followed by AC is effective, but the addition of capecitabine to standard docetaxel causes increased, although non–life threatening, toxicity. As a result, we do not believe that this regimen warrants further investigation in the neoadjuvant setting. This is in keeping with the philosophy that the risks of toxicity should be weighed against the benefits when considering breast cancer adjuvant therapy, because many such patients have excellent prognoses.

We hypothesized that baseline TP expression would correlate with the efficacy of DC chemotherapy, as suggested by preclinical models. Xenograft models have also shown that taxanes induce increased TP expression with peak levels observed 6 to 8 days after administration (7), thereby increasing the efficacy of capecitabine. However, no clinical study published to date has prospectively evaluated breast tumors after taxane therapy to determine if enzyme up-regulation occurs clinically. We used a novel dosing schedule, administering non-BSA–adjusted capecitabine 5 days after the first docetaxel dose to maximize patient exposure to capecitabine during the time of expected increase in TP. We did not observe consistent up-regulation of TP expression after docetaxel administration nor correlation between the level of TP expression and pathologic response. Our findings contrast with a recently reported study of patients with metastatic colorectal cancer that suggested the presence of TP expression at baseline was associated with better response to capecitabine and irinotecan compared with non-TP–expressing tumors (23). However, that report looked at absence or presence of TP expression and did not show whether the levels of expression correlated with response.

Although it is possible that TP expression was not optimally measured, we do not believe that our findings reflect this. Preclinical studies have used ELISA, which is not clinically practical given the requirement for a significant amount of fresh tissue, and immunohistochemistry to quantify TP expression (15). Success of our trial relied on accurate quantification of TP expression using paraffin-embedded tumor specimens. We have successfully used QIF to measure CYP3A4/5 expression in osteosarcoma samples (24) and, more recently, to measure TP expression in breast carcinoma samples. We independently used the previously validated immunohistochemistry method in addition to QIF to quantify TP. Because both methods yielded consistent results, we believe that our QIF results were accurate and may actually be more reliable than standard immunohistochemical staining.

Because this was a pilot study with small patient numbers, it is difficult to draw firm conclusions from the findings. For example, the significant toxicity that we encountered may not be observed in larger studies. Our therapy was similar to the Nadella regimen, which used the same dosing and timing of docetaxel, with capecitabine given on days 5 to 18 of a 28-day cycle (11), compared with administration on days 5 to 21 in this study. Phase I data revealed a maximum tolerated dose of capecitabine 1,250 mg/m^2/d (11). Based on prior pharmacokinetic data showing no change in clearance of capecitabine with changes in BSA (25), we used fixed-dose capecitabine in which patients were given 2,000 mg daily in two divided doses regardless of BSA. Prior experience at our institution has shown fixed dosing to be both convenient and feasible (26). The Nadella regimen used a dosing scheme of capecitabine based on the patient BSA, and would have resulted in an average dose of 2,375 mg, assuming an average BSA of 1.9 m^2 and the maximum tolerated dose of 1,250 mg/m^2/d for 14 days compared with the 17 days in the present study. The Nadella study was done on heavily pretreated patients with refractory disease. Still, we observed unacceptable toxicity rates despite

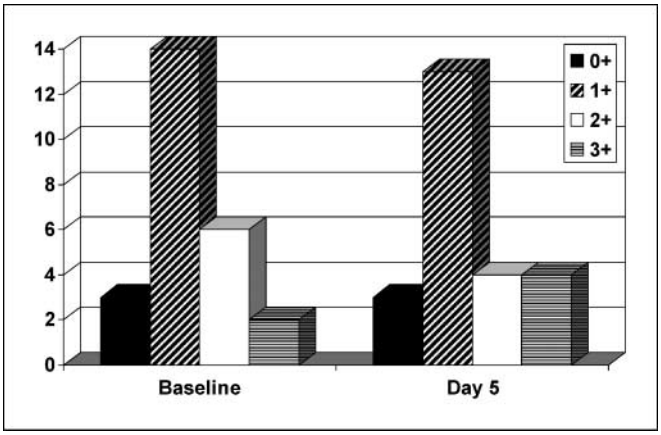

Fig. 4. TP measurement by immunohistochemistry before and after docetaxel. Number of patients with each immunohistochemical staining pattern of TP expression shown at baseline and 5 d after the first dose of docetaxel, demonstrating no significant TP up-regulation after docetaxel.

using a generally healthy patient population in the adjuvant setting, although the toxicity was not life threatening.

Although the results of this study did not corroborate our hypothesis, the unique study design provides an excellent framework for future neoadjuvant trials, which can facilitate efficient evaluation of new therapies. pCR has been endorsed as an effective surrogate marker for long-term disease-free survival (27, 28). Accordingly, neoadjuvant trials can generate reliable results within months compared with the many years of follow-up required for classic adjuvant studies. One of the criticisms of neoadjuvant chemotherapy is the incomplete and ambiguous staging before treatment. We and others have taken measures to optimize pretreatment lymph node staging through the use of sentinel lymph node biopsy in clinically node-negative patients (29–32) and the use of ultrasound-guided biopsy of clinically suspicious nodes. Because of the participation of breast surgeons, radiologists, radiation oncologists, pathologists, and medical oncologists in the conduct of this trial, we were able to perform and analyze 96% of patients' serial biopsies for TP. Additionally, the radiology-directed core biopsies obtained after neoadjuvant chemotherapy were able to accurately predict pCR in 88% of the cases. We plan to use this approach in future neoadjuvant studies to evaluate pCR and to obtain tissue for future correlative studies.

Acknowledgments

We thank Matthew H. Innes and Janet H. Tarolli, from the University of Michigan Comprehensive Cancer Center Clinical Trials Office, and Jacqueline H. Sheffield for their assistance.

References

1. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 1997;15:2483–93.
2. Kaufmann M, Hortobagyi GN, Goldhirsch A, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update. J Clin Oncol 2006;24:1940–9.
3. von Minckwitz G, Raab G, Caputo A, et al. Doxorubicin with cyclophosphamide followed by docetaxel every 21 days compared with doxorubicin and docetaxel every 14 days as preoperative treatment in operable breast cancer: the GEPARDUO study of the German Breast Group. J Clin Oncol 2005;23:2676–85.
4. Fisher B, Brown AM, Dimitrov NV, et al. Two months of doxorubicin-cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate, and fluorouracil in positive-node breast cancer patients with tamoxifen-nonresponsive tumors: results from the National Surgical Adjuvant Breast and Bowel Project B-15. J Clin Oncol 1990;8:1483–96.
5. Henderson IC, Berry DA, Demetri GD, et al. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 2003;21:976–83.
6. Citron ML, Berry DA, Cirrincione C, et al. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. J Clin Oncol 2003;21:1431–9.
7. Sawada N, Ishikawa T, Fukase Y, Nishida M, Yoshikubo T, Ishitsuka H. Induction of thymidine phosphorylase activity and enhancement of capecitabine efficacy by Taxol/Taxotere in human cancer xenografts. Clin Cancer Res 1998;4:1013–9.
8. Fujimoto-Ouchi K, Tanaka Y, Tominaga T. Schedule dependency of antitumor activity in combination therapy with capecitabine/5′-deoxy-5-fluorouridine and docetaxel in breast cancer models. Clin Cancer Res 2001;7:1079–86.
9. Grem J. 5-Fluoropyrimidines. In: Chabner B, Longo D, editors. Cancer chemotherapy and biotherapy. 2nd ed. Philadelphia: Lippincott-Raven Publishers; 1996. p. 149-211.
10. O'Shaughnessy J, Miles D, Vukelja S, et al. Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. J Clin Oncol 2002;20:2812–23.
11. Nadella P, Shapiro C, Otterson GA, et al. Pharmacobiologically based scheduling of capecitabine and docetaxel results in antitumor activity in resistant human malignancies. J Clin Oncol 2002;20:2616–23.
12. Salonga D, Danenberg KD, Johnson M, et al. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. Clin Cancer Res 2000;6:1322–7.
13. Fox SB, Westwood M, Moghaddam A, et al. The angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase is upregulated in breast cancer epithelium and endothelium. Br J Cancer 1996;73:275–80.
14. Leek RD, Landers R, Fox SB, Ng F, Harris AL, Lewis CE. Association of tumour necrosis factor α and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer 1998;77:2246–51.
15. Kurosumi M, Sugamata N, Tabei T, et al. Comparison of ELISA level and immunohistochemical status for thymidine phosphorylase (TP) in invasive breast carcinoma. Anticancer Res 2002;22:331–8.
16. Yang Q, Barbareschi M, Mori I. Prognostic value of thymidine phosphorylase expression in breast carcinoma. Int J Cancer 2002;97:512–7.
17. Takahashi H, Maeda Y, Watanabe K, Taguchi K, Sasaki F, Todo S. Correlation between elevated intratumoral thymidine phosphorylase and prognosis of node-positive breast carcinoma undergoing adjuvant doxifluridine treatment. Int J Oncol 2000;17:1205–11.
18. Ishikawa T, Sekiguchi F, Fukase Y, Sawada N, Ishitsuka H. Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. Cancer Res 1998;58:685–90.
19. Tominaga T, Toi M, Ohashi Y, Abe O. Prognostic and predictive value of thymidine phosphorylase activity in early-stage breast cancer patients. Clin Breast Cancer 2002;3:55–64.
20. Wang S, Saboorian MH, Frenkel E, Hynan L, Gokaslan ST, Ashfaq R. Laboratory assessment of the status of Her-2/neu protein and oncogene in breast cancer specimens: comparison of immunohistochemistry assay with fluorescence *in situ* hybridisation assays. J Clin Pathol 2000;53:374–81.
21. Thall PF, Simon R. Practical Bayesian guidelines for phase IIB clinical trials. Biometrics 1994;50:337–49.
22. Bear HD, Anderson S, Brown A, et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2003;21:4165–74.
23. Meropol NJ, Gold PJ, Diasio RB, et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 2006;24:4069–77.
24. Dhaini HR, Thomas DG, Giordano TJ, et al. Cytochrome P450 CYP3A4/5 expression as a biomarker of outcome in osteosarcoma. J Clin Oncol 2003;21:2481–5.
25. Cassidy J, Twelves C, Cameron D, et al. Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients. Cancer Chemother Pharmacol 1999;44:453–60.
26. Schott A, Hayes DF, Stearns V, Wicha M, Baker L. Vinorelbine (VR) and capecitabine (CAP) in metastatic breast cancer: phase I/II study with correlative genotype, phenotype, and pharmacokinetics. Breast Cancer Res Treat 2002;76:S91.
27. Sataloff DM, Mason BA, Prestipino AJ, Seinige UL, Lieber CP, Baloch Z. Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of outcome. J Am Coll Surg 1995;180:297–306.
28. Bear HD, Anderson S, Smith RE, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2006;24:2019–27.
29. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. Lancet 1997;349:1864–7.
30. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994;220:391–8; discussion 398–401.
31. Giuliano AE, Jones RC, Brennan M, Statman R. Sentinel lymphadenectomy in breast cancer. J Clin Oncol 1997;15:2345–50.
32. Albertini JJ, Lyman GH, Cox C, et al. Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. JAMA 1996;276:1818–22.

Clinical Cancer Research

Neoadjuvant Docetaxel and Capecitabine and the Use of Thymidine Phosphorylase as a Predictive Biomarker in Breast Cancer

Rachel M. Layman, Dafydd G. Thomas, Kent A. Griffith, et al.

Clin Cancer Res 2007;13:4092-4097.