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Report

Randomized phase II trial of the anti-angiogenic potential of doxorubicin and docetaxel; primary chemotherapy as Biomarker Discovery Laboratory

Kathy D. Miller[1], Sharon E. Soule[1], Cynthia Calley[2], Robert E. Emerson[3], Gary D. Hutchins[4], Kenyon Kopecky[4], Sunil Badve[3], AnnaMaria Storniolo[1], Robert Goulet[5], and George W. Sledge, Jr[1]

[1]*Division of Hematology and Oncology;* [2]*Department of Biostatistics;* [3]*Department of Anatomic Pathology;* [4]*Department of Radiology;* [5]*Department of Surgery, Indiana University, Indianapolis, IN*

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Summary

Purpose: Primary chemotherapy provides an ideal opportunity to correlate potential non-invasive surrogate markers of angiogenesis with tumor microvessel density (MVD) and response. *Patients and methods*: Patients with newly diagnosed stages II or III breast cancer were treated with sequential doxorubicin 75 mg/M2 q2 wks × 3 and docetaxel 40 mg/M2 weekly × 6; treatment order was randomly assigned. Potential serologic and imaging markers of angiogenesis were obtained pre-treatment, at crossover and completion of chemotherapy. Non-invasive biomarkers were correlated with MVD and pathologic response. *Results*: From June 1999 to October 2002, 70 patients were entered. Median pretreatment tumor diameter was 6.0 cm with clinically involved axillary nodes in 33 (47%) patients; 20% had inflammatory disease. Clinical response rate was 91%, including 46% clinical complete responses. Pathologic complete response (pCR) was confirmed in 9 (12.8%) patients. Baseline MVD did not correlate with clinical or pathologic response. Serologic markers were obtained in all patients; basic fibroblast growth factor (bFGF) was lower at baseline and increased during treatment in patients with a pCR but did not correlate with MVD. Color Doppler ultrasound (CDUS) was completed in 47 patients; no parameter reliably correlated with MVD or response. Positron emission tomography (PET) with [F-18]-fluoro-deoxyglucose, [O-15]-water and [C-11]-carbon monoxide were completed in 19 patients; uptake of all tracers decreased during treatment in virtually all patients. *Conclusion:* Sequential doxorubicin and docetaxel is generally well tolerated and highly active. Serum angiogenic factors and imaging parameters frequently varied throughout treatment but did not correlate with MVD or consistently predict response.

Introduction

Growth and metastasis of solid tumors depends on angiogenesis, the formation of new blood vessels to nourish the tumor [1]. Both laboratory and indirect clinical evidence support the central role of angiogenesis in breast cancer progression (reviewed by Gasparini [2]). The central importance of angiogenesis has led to novel therapies designed to interrupt this process; several specific anti-angiogenic agents are currently in clinical trials either as monotherapy or in combination with traditional cytotoxic agents. The intense interest in angiogenesis has also led to a re-examination of the activity of many established cytotoxic agents. Indeed, a number of existing chemotherapy agents, including the taxanes and anthracyclines, have distinct anti-angiogenic activity [3]. The taxanes inhibit endothelial proliferation, migration and tubule formation at levels significantly below those required for tumor cell kill [4–6]. Non-cytotoxic doses of doxorubicin selectively inhibit endothelial proliferation, decrease collagenase I gene expression and reduce the ability of tumor cells to invade a collagen matrix independent of any anti-proliferative effect [7, 8].

Successful development of anti-angiogenic agents requires new approaches to therapy and clinical research: biologically active rather than maximal tolerated dose, chronic rather than intermittent therapy, induction of tumor dormancy or control rather than tumor cell kill. Correlative laboratory studies assessing biologically meaningful intermediate endpoints are a necessity. Despite the wealth of laboratory data, direct clinical evidence linking anti-angiogenic activity, intermediate endpoints, changes in tumor microvessel

density (MVD) and objective tumor response is lacking. Though as yet no clear standard has emerged, the search for reliable surrogates of anti-angiogenic activity has focused on two main areas: soluble factors and imaging the tumor vasculature.

Randomized trials have confirmed the safety and activity of primary chemotherapy for breast cancer. Though disease-free and overall survival are unchanged, primary chemotherapy is effective in down-staging tumors, increasing both the proportion of patients with negative lymph nodes and the ability to perform breast-conserving surgery [9–12]. The extent of residual tumor after primary chemotherapy correlates tightly with overall survival [13–15]. Despite significant tumor down-staging with primary chemotherapy, only a small fraction of patients obtain a pathologic complete remission; paired pre- and post-treatment tissue samples are available for the majority of patients. Prognostic factors including MVD can be determined by core needle biopsy. This trial was designed to exploit the unique potential of primary chemotherapy to assess the clinical relevance of the anti-angiogenic activity of cytotoxic therapy and to correlate potential non-invasive surrogate markers of angiogenesis with tumor MVD and response.

Patients and methods

Eligibility criteria

Patients with histologically confirmed stages II or III breast cancer who had not undergone definitive surgical resection were eligible. Patients were required to have disease measurable by physical examination or diagnostic breast imaging with a primary tumor ≥2 cm. Prior breast or chest wall radiation, chemotherapy or hormonal therapy was not allowed. Patients had to have adequate renal, hepatic, hematologic and cardiac function. The Indiana University institutional review board approved the protocol and patients provided written informed consent prior to treatment.

Treatment plan

After eligibility was confirmed, patients received sequential doxorubicin (A) 75 mg/M^2 every 2 weeks for three cycles and docetaxel (T) 40 mg/M^2 weekly for six cycles (Figure 1). Treatment order (A > T versus T > A) was randomly assigned after stratification for tumor size (≤5 cm versus > 5 cm) and clinical axillary node status (positive versus negative). Granulocyte colony stimulating factor (G-CSF) was administered as a once daily subcutaneous injection on days 2–11 of each doxorubicin treatment cycle; G-CSF was not routinely administered after docetaxel treatment. Dexamethasone, 4 mg orally every 12 h for three doses, was administered starting 12 h prior to each docetaxel treatment to prevent fluid retention.

Dose modifications were based on nadir blood counts and interval toxicity. Doxorubicin was held if neutrophils were <1200/mm^3 or platelets were <100,000/mm^3. Docetaxel was held if neutrophils were <1000/mm^3 or platelets were <75,000/mm^3; docetaxel dose was reduced 50% if neutrophils were 1000–1199/mm^3 or platelets were 75,000–99,000/mm^3. A 25% dose reduction was mandated for any of the following: fever associated with neutrophils <1000/mm^3, infection requiring hospitalization or parenteral antibiotics, bleeding associated with platelet count <40,000/mm^3, grade 3 or 4 mucositis; grade 3 or 4 diarrhea mandated a 75% dose reduction. Patients were evaluated for disease response after 6 and 12 weeks of therapy. Diagnostic breast imaging (mammogram or ultrasound) was repeated prior to definitive surgery in all patients; extent of surgery was left to the discretion of the treating oncologic surgeon. Postoperative chemotherapy and radiation was administered at the discretion of the treating medical oncologist; tamoxifen was recommended for all patients with ER positive tumors. In keeping with the objectives of this study, patients were not followed for recurrence or survival.

Microvessel density

MVD was assessed using a previously published method [16]. Briefly, core biopsy and definitive surgical specimens were stained with CD31 and scanned at low power to identify the three areas of greatest vessel density. These areas were then examined under high power (200×); any brown-staining endothelial cell clearly separate from the surrounding microvasculature was counted. Both the maximum and average of all three fields were reported. A single pathologist (REE), blinded

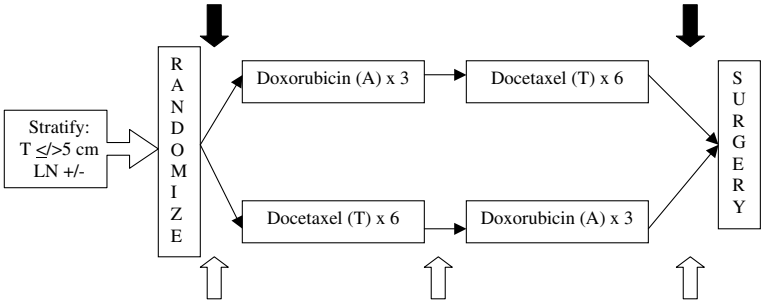

Figure 1. Study schema. Open arrows indicate timing of clinical tumor assessment, serum collection and imaging. Filled arrows indicate timing of histology and MVD measures.

to treatment assignment and other surrogate marker results, evaluated all samples.

Serum angiogenic factors

Serum samples were collected pre-treatment, prior to chemotherapy on week 7 and at completion of all chemotherapy (prior to surgery). Samples were obtained in a serum separator or standard red top tube and allowed to clot on ice for 30 minutes, then separated by centrifugation at $3000 \times g$ for 30 min. Serum was cryopreserved at −20 °C in 1 ml aliquots for later analysis; at least three aliquots were preserved for each patient at each time point. Serum vascular cell adhesion molecule-1 (VCAM-1), bFGF, matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) were measured in duplicate using commercially available enzyme linked immunosorbant assays (R & D Systems and Oncogene Research Products). All samples with a coefficient of variation > 10% were repeated. The assays have the following limits of detection: VCAM1 – < 2 ng/ml, bFGF – < 1 ng/ml, MMP-2 – < 0.37 ng/ml, MMP-9 – < 0.156 ng/ml.

Color Doppler ultrasound (CDUS)

CDUS was performed pre-treatment, prior to chemotherapy on week 7 and at completion of all chemotherapy; complete imaging data is available for 47 patients. CDUS was performed using a 5 MHz transducer and 3.5 MHz pulsed color-coded Doppler (HDI 5000; Advanced Technology Laboratories, Bothell, WA). Following identification of the tumor by standard B-mode techniques, areas of interest in the tumor and immediately adjacent tissues were scanned with color Doppler in different planes to assess vascularity; multiple data sets were obtained from all parts of the tumor, including the tumor periphery. Systolic and diastolic diameters of the largest visible vessel in the tumor were measured. Recording frequency spectra in the Duplex mode completed the ultrasound study. After adjusting the angle between the ultrasound beam and the blood flow vector, Doppler frequency spectra were analyzed for peak systolic (V_{max}) and end diastolic velocities (V_{ED}) in the largest visible vessel in the tumor; this system allows detection of flow velocities as slow as 0.3 cm/s. In addition, the time average mean velocity (TAM) and mean blood flow (limit = 0.1 ml/min) were measured. Resistive index (RI) was calculated as $(V_{max}-V_{ED})/V_{max}$. To reduce variability, the same technician performed all CDUS examinations; a single radiologist (KK), blinded to treatment assignment and other surrogate marker results, reviewed all CDUS images.

Positron emission tomography (PET)

PET images with [F-18]-fluoro-deoxyglucose (FDG), [O-15]-water (H2O) and [C-11]-carbon monoxide (CO) were obtained pre-treatment, prior to chemotherapy on week 7 and at completion of all chemotherapy in 28 patients; complete data for all tracers and time points is available for 19 patients.

Region-of-interest (ROI) and index definition

Quantitative indices of glucose utilization, tumor blood flow and tumor blood volume were calculated based upon the measured kinetics of [F-18]FDG, [O-15]H2O and [C-11]CO, respectively. In each study, a circular ROI was drawn around the tumor in the FDG image (45–60 min post injection) on each image plane in which the tumor was visible. An automated routine then scanned a 5×5 ROI, centered on each voxel within the circular ROIs to identify a single region of maximal FDG retention. The square region of maximal FDG retention was then applied to the blood flow and blood volume images to obtain estimates for the same tissue region as the FDG estimate. In the contralateral breast, a series of circular ROIs were drawn in each image plane that contained breast tissue. A 5×5 pixel ROI was scanned throughout each circular ROI to obtain maximum ROI values. The mean and standard deviation of the maximum ROI values in the contralateral breast were then calculated for each study. A tracer uptake index was calculated for each study by dividing the difference between the maximal tumor uptake and the mean contralateral breast uptake by the standard deviation of the contralateral breast uptake values. That is, $UI = (tumor_{max} - \text{mean } breast_{max}) / \text{standard deviation } breast_{max}$.

Glucose utilization

Estimates of glucose utilization were generated by integrating the FDG uptake over the time period of 45–60 min post injection of 10 mCi of [F-18]FDG. The FDG integral image was divided by the integral of [F-18] in the arterial blood, obtained by placing an ROI over the left ventricular bloodpool of the heart. Calculations were generated on a pixel by pixel basis to generate parametric images of FDG retention.

Tumor blood flow

Estimates of tumor blood flow were obtained by fitting a 2-compartment model for freely diffusible tracers to the uptake and washout kinetics of [O-15]H2O after injection of 50 mCi of [O-15]H2O. The input function for the model was obtained by placing an ROI over the left ventricular bloodpool of the heart. Blood flow estimates were generated on a pixel by pixel basis to generate parametric blood flow images.

Tumor blood volume

Estimates of tumor blood volume were generated by integrating the [C-11]CO uptake over the time period of 5–20 min post injection of 30 mCi of [C-11]CO. Blood volume estimates were obtained by dividing the integrated CO image by the integral of the [C-11]CO activity observed in the left ventricle bloodpool of the heart.

Definition of response

Clinical complete response (cCR) was defined as the complete disappearance of all evidence of disease by physical examination. Clinical partial response (cPR) required at least a 50% decrease in the product of the greatest perpendicular tumor diameters. Pathologic complete response (pCR) required no evidence of invasive malignancy in the breast and lymph node specimens at the time of definitive surgery.

Statistical considerations

Sample size calculations were based on toxicity, considering a 40% incidence of ≥grade 4 neutropenia or ≥grade 3 non-hematologic toxicity unacceptable. As treatment sequence may impact toxicity, each arm was considered independently. Each arm proceeded with a two-stage design. The first stage enrolled 13 patients; if <5 patients had unacceptable toxicity enrollment proceed to a total of 35 patients per arm. This design provided 80% power (alpha = 0.05) for each arm. Confidence interval for true toxicity rate was calculated using the method of Jennison and Turnbull [17]. Correlation between potential non-nvasive biomarkers and pCR or MVD were analyzed using *t*-test (to compare the sample means of responders and non-responders) and ANOVA (to compare sample means among all response status groups).

Results

Clinical data

From June 1999 to October 2002, 70 patients were enrolled. Initial patient characteristics were well matched (Table 1). The median age was 50 ears (range 30–65). Median pretreatment tumor size was 6.0 m with clinically positive axillary lymph nodes in 33 (47%) patients; 14 (20%) patients had inflammatory disease. All patients were evaluable for toxicity and pathologic response; clinical response could not be accurately assessed in one patient with diffuse inflammatory disease but no discretely measurable tumor.

Overall treatment was well tolerated (Table 2). Myelosuppression was uncommon and rarely complicated by infection. Anemia and thrombocytopenia were generally mild; transfusions were not required. Nausea was the most commonly reported toxicity during doxorubicin therapy. Fatigue was most common during treatment with docetaxel; hand-foot syndrome and persistent tearing were infrequent. The toxicity of both drugs tended to be slightly more prominent when administered second. Nonetheless, excellent dose intensity was maintained.

Objective clinical responses (cCR + cPR) were obtained in 63 (91%) patients; 32 (46.4%) patients obtained a cCR (Table 3). Altogether a pCR was confirmed in 9 (12.8%) patients. Clinical response rate was similar irrespective of treatment order. Though slightly more patients receiving docetaxel followed by doxorubicin achieved a pCR, this trend did not reach statistical significance. Thirty-seven (52.8%) patients had pathologic negative nodes.

Table 1. Patient and tumor characteristics

	Total (n = 70)	A > T (n = 35)	T > A (n = 35)
Median age	50 (30–65)	50 (36–65)	50 (30–64)
Median tumor size	6.0 cm	6.0 cm	5.5 cm
Inflammatory disease	14 (20%)	8 (23%)	6 (17%)
Palpable lymph nodes	33 (47%)	17 (49%)	16 (46%)
ER+	40 (57%)	20 (57%)	20 (57%)
HER2+ (IHC 3+ or FISH+)	14 (20%)	6 (17%)	8 (23%)

ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, FISH = fluorescence *in situ* hybridization.

Pre-treatment tumor sample was inadequate to quantify MVD in 10 (14%) patients, 5 in each treatment arm. Baseline MVD was highly variable with a 40-fold range between the tumors with the highest and lowest recorded MVDs (Table 4). In addition to the nine patients with a pCR, nine additional patients had insufficient residual tumor at the time of definitive surgery for MVD analyses. Pre- and post-treatment MVD were similar in most of the 46 patients for whom paired samples were available (Figure 2).

Correlative studies

VCAM-1, MMP-2 and MMP-9 were highly variable at diagnosis but baseline levels did not predict response. Similarly, changes in serum concentrations during therapy were not significantly different between responding and non-responding patients. In contrast, bFGF was significantly lower at diagnosis ($p = 0.0227$) in patients who ultimately achieved a pCR; bFGF levels tended to increase during treatment in patients with a pCR but remained stable in patients without a pCR (Figure 3).

CDUS imaging was completed in 47 patients, including 6 who achieved ultimately achieved a pCR (Table 5). Vessels remained visible by CDUS in 2 of 6 patients with a pCR at the time of surgery. No vessels were identified in 11/41 (26.8%) with residual disease; four had only scattered viable tumor cells and two had residual disease only in the axillary nodes.

PET imaging was conducted in 28 but complete data for all tracers and time points was obtained in only 19 patients, including 3 who achieved ultimately achieved a pCR (Figure 4). When compared to the unaffected breast, glucose utilization and tumor blood flow were similar in responding and non-responding patients; both decreased during therapy in virtually all patients,

Table 2. Toxicity and dose intensity

	A > T (n = 35)				T > A (n = 35)			
	A		T		T		A	
	G3(%)	G4(%)	G3(%)	G4(%)	G3(%)	G4(%)	G3(%)	G4(%)
Myelosuppression								
Neutropenia	0	0	4 (11)	0	1 (3)	1 (3)	0	2 (6)
Anemia	0	0	0	0	0	0	1 (3)	0
Infection	0	0	0	0	0	0	2 (6)	1 (3)
Gastrointestinal								
Nausea	2 (6)	0	1 (3)	0	2 (6)	0	5 (14)	0
Anorexia	0	0	0	0	1 (3)	0	1 (3)	0
Mucositis	2 (6)	0	0	0	0	0	0	0
Diarrhea	2 (6)	1 (3)	2 (6)	0	3 (9)	0	2 (6)	0
Constipation	1 (3)	0	1 (3)	0	0	0	0	0
Fatigue	2 (6)	0	5 (14)	0	2 (6)	1 (3)	1 (3)	0
Hand-foot syndrome	0	0	2 (6)	0	1 (3)	0	0	0
Anxiety/depression	1 (3)	0	2 (6)	0	1 (3)	0	2 (6)	0
Tearing	0	0	0	0	1 (3)	0	0	0
Dose intensity[a]	95.2%		89.2%		97.2%		94.2%	

National Cancer Institute Common Toxicity Criteria (version 2.0) worst grade experienced per patient.
[a]Calculated as % of planned dose intensity delivered.

Table 3. Clinical and pathological response

	Total (n = 69)	A > T (n = 35)	T > A (n = 34)
Clinical response			
cCR	32 (46.4%)	14 (40%)	18 (53%)
cPR	31 (45%)	18 (51%)	13 (38%)
cSD	5 (7.2%)	3 (9%)	2 (6%)
cPD	1 (1.4%)	0	1 (3%)
Pathological response	Total (n = 70)	A > T (n = 35)	T > A (n = 35)
pCR	9 (12.8%)	3 (8.6%)	6 (17.1%)
Mean tumor size (cm)	2.0	2.3	1.7
Involved lymph nodes			
0	37 (52.9%)	18 (51.4%)	19 (54.3%)
1–3	20 (28.6%)	10 (28.6%)	10 (28.6%)
4–9	12 (17.1%)	6 (17.1%)	6 (17.1%)
≥10	1 (1.4%)	1 (2.9%)	0
Breast conservation	26 (37%)	12 (34%)	14 (40%)

Table 4. Tumor MVD

	Total	A > T	T > A
Diagnosis			
Mean maximum MVD (/mm^3)	224 (24–957) (n = 60)	223 (24–676) (n = 30)	225 (24–957) (n = 30)
Mean mean MVD (/mm^3)	172 (17–498) (n = 60)	171 (19–430) (n = 30)	172 (17–498) (n = 30)
Definitive surgery			
Mean maximum MVD (/mm^3)	256 (58–837) (n = 52)	236 (58–593) (n = 27)	277 (89–837) (n = 25)
Mean mean MVD (/mm^3)	203 (45–543) (n = 52)	189 (45–537) (n = 27)	218 (77–543) (n = 25)

regardless of response. In contrast, tumor blood volume was slightly lower at baseline in patients with a pCR; tumor blood volume remained unchanged in patients with a pCR but tended to increase in patients without a pCR. Given the small number of patients with complete PET imaging data, none of these differences reached statistical significance. Treatment order did not influence PET imaging results; there were no associations between uptake of [F-18]-FDG, [O-15]-H2O or [C-11]-CO (data not shown).

None of the measured serum proteins or imaging parameters consistently correlated with MVD (Table 6). Patient demographics, tumor characteristics and MVD at diagnosis did not predict pathologic response; only lower serum bFGF concentration at diagnosis was associated with pCR (Table 7). Given the small number of patients with a pCR and lack of significant associations in univariate analyses, multivariate analyses were not conducted.

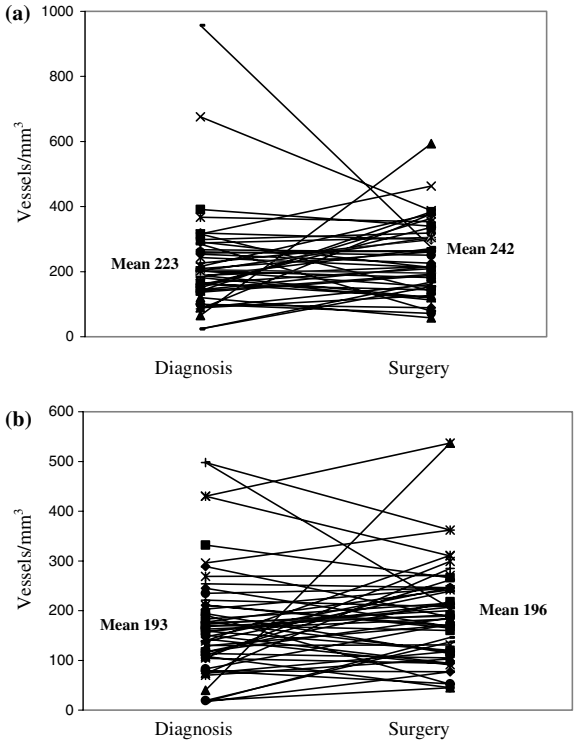

Figure 2. Maximum (a) and average (b) tumor MVD at diagnosis and definitive surgery for the 46 available paired samples.

Discussion

Primary chemotherapy with sequential dose-dense doxorubicin and weekly docetaxel is generally well tolerated and highly active. Severe hand-foot syndrome, which frequently limited therapy in regimens using sequential doxorubicin followed docetaxel administered on an every 2-week schedule [18–20], was not encountered. Given the limited duration of weekly docetaxel treatment, only one patient developed significant tearing [21,22]. Despite the decrease in toxicity, the pathologic complete remission rate is identical to our previous trial [20] and similar to that reported by other investigators using dose-dense regimens in patients with locally advanced disease [23–26]. Consistent with the recently reported E1193 trial comparing combination to sequential doxorubicin and paclitaxel [27], treatment order did not impact response.

In our study, only lower serum bFGF concentration at diagnosis predicted a greater likelihood of pCR. No other single factor, whether assessed at baseline or dynamically during treatment, predicted pCR. Though the improvement in response in patients with lower bFGF levels at diagnosis did reach statistical significance, caution is required in interpreting these data. First, these results conflict with the study reported by Linderholm et al. [28] in which patients with higher tumor cytosolic bFGF levels enjoyed a lower recurrence rate and improved overall survival. Secondly, we must caution that the association between lower baseline bFGF and response in our patients may be spurious, resulting merely from the small sample size and multiple comparisons [29,30].

Previous attempts to identify tumor characteristics that predict response to primary chemotherapy have yielded mixed and at time contradictory results. Several investigators have reported an increased likelihood of response in patients with ER negative tumors [31–33] while others report a trend toward higher response rates to neoadjuvant chemoendocrine therapy in ER positive tumors [34,35]. Similarly conflicting results have been reported for the association of HER2 expression, p53

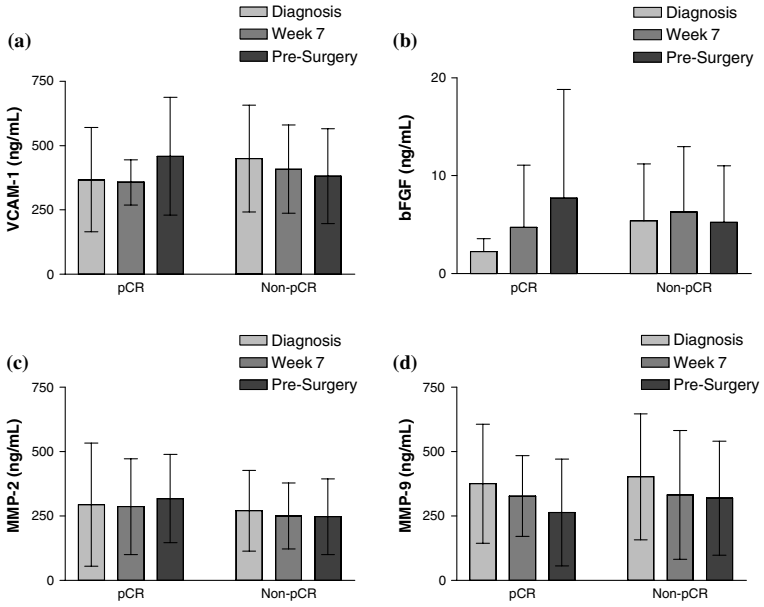

Figure 3. Mean serum angiogenic protein levels at baseline and during therapy for patients achieving a pCR ($n = 9$) versus patients without a pCR ($n = 61$). (a) VCAM-1: baseline pCR = 368 ± 203 ng/ml; baseline non-pCR = 450 ± 207, (b) bFGF: baseline pCR = 2.28 ± 1.3 ng/ml; baseline non-pCR = 5.43 ± 5.8, (c) MMP-2: baseline pCR = 293 ± 238 ng/ml; baseline non-pCR = 270 ± 156 (d) MMP-9: baseline pCR = 375 ± 231 ng/ml; baseline non-pCR = 402 ± 244.

Table 5. CDUS Imaging Parameters[a]

	Diagnosis	Week 7	Pre-Surgery
V_{max} (cm/s)			
pCR ($n = 6$)	11.18 ± 10.77	11.17 ± 13.21	3.73 ± 6.01
Non-pCR ($n = 41$)	17.72 ± 20.64	11.73 ± 12.67	9.21 ± 11.43
V_{ED} (cm/s)			
pCR	2.83 ± 2.83	2.17 ± 3.54	1.02 ± 1.58
Non-pCR	4.91 ± 4.78	3.93 ± 4.11	3.47 ± 4.15
Resistive index			
pCR	0.66 ± 0.20	0.58 ± 0.38	0.28 ± 0.39
Non-pCR	0.67 ± 0.19	0.59 ± 0.23	0.39 ± 0.31
Mean blood flow (ml/min)			
pCR	1.55 ± 2.62	0.3 ± 0.5	0.33 ± 0.55
Non-pCR	3.25 ± 3.49	1.75 ± 2.17	1.33 ± 2.01
Max. systolic diameter (mm)			
pCR	0.85 ± 0.69	0.72 ± 0.74	0.37 ± 0.58
Non-pCR	1.11 ± 0.56	0.56 ± 0.41	1.33 ± 2.01
Max. diastolic diameter (mm)			
pCR	0.65 ± 0.57	0.53 ± 0.59	0.30 ± 0.48
Non-pCR	0.80 ± 0.53	0.60 ± 0.36	0.89 ± 2.30

[a]Mean and standard deviation for each parameter.

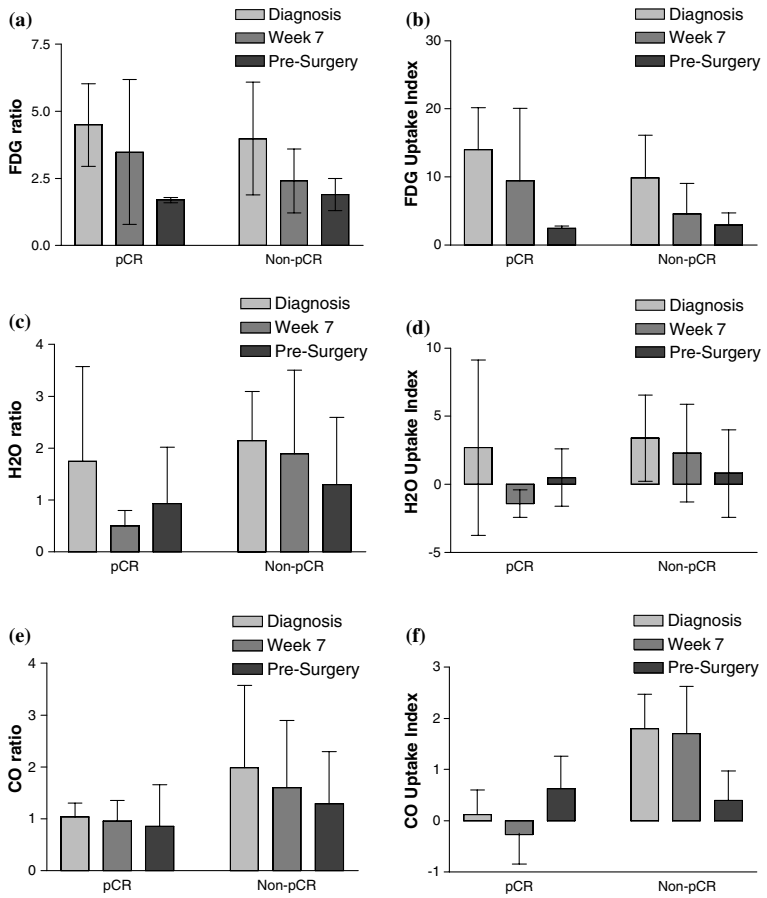

Figure 4. PET imaging parameters at baseline and during therapy for patients achieving a pathologic complete response ($n = 3$) versus patients without a pCR ($n = 16$). (a) [F-18]-FDG ratio, (b) [F-18]-FDG UI (c) [O-15]-H2O ratio, (d) [O-15]-H2O UI, (e) [C-11]-CO ratio, (f) [C-11]-CO UI.

Table 6. Association with MVD*

	Diagnosis	Surgery
Serum factors		
VCAM-1	0.0990	0.3462
bFGF	0.1384	0.0567
MMP-2	0.5714	0.0472
MMP-9	0.4317	0.8018
CDUS parameters		
Peak systolic velocity	0.2275	0.9072
End diastolic velocity	0.2377	0.9873
RI	0.9565	0.0698
TAM velocity	0.3009	0.8644
Mean blood flow	0.3591	0.4198
Maximum systolic diameter	0.0362	0.1959
Maximum diastolic diameter	0.1680	0.5814
PET parameters		
[F-18]FDG ratio	0.0662	0.4082
[F-18]FDG UI	0.0831	0.3305
[O-15]H2O ratio	0.6495	0.6797
[O-15]H2O UI	0.7841	0.7801
[C-11]CO ratio	0.5523	0.3186
[C-11]CO UI	0.9330	0.3107

*Univariate *p*-values for association with average MVD at time of diagnosis (initial biopsy) and definitive surgery.

Table 7. Association with complete pathologic response*

	Diagnosis	Change during treatment
Age	0.790	NA
Tumor characteristics		
Initial tumor size	0.479	NA
ER	0.9368	NA
HER2	0.0744	NA
MVD at diagnosis	0.2676	NA
Serum factors		
VCAM-1	0.0848	0.2028
bFGF	0.0227	0.2003
MMP-2	0.5481	0.3841
MMP-9	0.5062	0.2544
CDUS parameters		
Peak systolic velocity	0.6188	0.9229
End diastolic velocity	0.5941	0.9679
RI	0.3517	0.5300
TAM velocity	0.9877	0.7063
Mean blood flow	0.6692	0.6215
Maximum systolic diameter	0.5104	0.7894
Maximum diastolic diameter	0.5941	0.7456
PET parameters		
[F-18]FDG ratio	0.3364	0.7396
[F-18]FDG UI	0.6613	0.8676
[O-15]H2O ratio	0.9824	0.6580
[O-15]H2O UI	0.8533	0.9935
[C-11]CO ratio	0.6508	0.4024
[C-11]CO UI	0.3821	0.4642

*Univariate *p*-values for association with complete pathologic response.

mutations, tumor grade, bcl-2 and proliferation rate with response to primary chemotherapy [31,34–38]. Differences in tissue fixation, staining methods, analytic criteria and interobserver variability among pathologists surely account for some of these disparate results [39–41].

Similarly, we could not identify any CDUS or PET imaging parameter that reliably predicted response. Kedar et al. serially evaluated 34 patients receiving primary chemotherapy with CDUS and traditional B-mode ultrasonography. CDUS frequently identified responding patients before decreases in tumor size were apparent by physical examination; correlation with pathologic response was not reported [42].

Several investigators have reported that early decreases in FDG or technetium 99m-sestamibi uptake distinguished clinically responding from non-responding patients [43–47]. Smith et al. [48] correlated FDG uptake with pathologic response in 31 primary breast tumors. The mean pretreatment FDG uptake ratio of the eight tumors that achieved a complete pathologic response in the breast were significantly ($p = 0.037$) higher than those from less responsive lesions; of note two of these eight patients had residual lymph node disease at the time of surgery. Though FDG imaging identifies patients responding by clinical criteria, we suggest it is not sufficiently discriminatory to identify patients with a pCR by our strict definition.

We had also hoped to identify a surrogate marker for tumor MVD. Unfortunately, none of the angiogenic peptides or imaging parameters we employed reliably correlated with MVD. Our results are consistent with other investigators who have failed to find a significant correlation between CDUS parameters and MVD [49,50]. We hypothesize CDUS assesses the macrovasculature or 'feeder vessels' of the tumor while MVD assesses the microvascular, capillary network.

Bos et al. compared FDG uptake with tumor histology in 55 primary breast tumors. FDG uptake significantly correlated with Glut-1 expression ($p < 0.001$), mitotic activity index ($p = 0.001$), tumor necrosis ($p = 0.010$), tumor cells/volume ($p = 0.009$), hexokinase I expression ($p = 0.019$), lymphocyte infiltration ($p = 0.032$) and MVD ($r = 0.373$; $p = 0.0050$) [51]. In two studies FDG uptake correlated with tumor blood flow as assessed by [O-15]H2O but MVD was not measured [52–54]. The small number of patients who completed all PET imaging studies limited our ability to confirm a correlation with MVD.

Many tumor characteristics can be reliably assessed in core biopsy specimens [55], but the tumor vasculature is heterogenous with the highest MVD typically found

at the tumor periphery. Even with image guidance, the core biopsy may not contain the most vascular area of the tumor. Jacobs et al. compared MVD in 49 paired core biopsy and excised tumor specimens. Although there was significant correlation between MVD measurements ($r = 0.507$, $p = 0.0002$), the mean MVD on the core biopsy and corresponding excision specimens differed by more than 10% in 85.7% of cases, with differences ranging from 4.3 to 233.3% [56].

Given the complex biology underlying response to chemotherapy, it now seems naïve to expect any *single* factor to be sufficiently predictive to guide therapy. Approaches utilizing gene array technology, which allow simultaneous interrogation of multiple genes, may be more fruitful [57–59]. RNA was extracted from fixed tissue and subjected to gene array analysis in a subset of patients enrolled in this trial ($n = 45$). Full results will be reported separately but preliminary analysis of 192 candidate genes identified 19 that were differentially expressed in patients achieving a PCR [60].

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Address for offprints and correspondence: Kathy D. Miller, 535 Barnhill Drive, RT-473, Indianapolis, IN 46202, USA; *Tel.*: +1-317-274-0920; *Fax*: +1-317-274-3646; *E-mail:* kathmill@iupui.edu