A Vascular Targeting Regimen of Preoperative Docetaxel with or without Bevacizumab for Locally Advanced Breast Cancer: Impact on Angiogenic Biomarkers

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Abstract

Purpose: Taxanes have effects on angiogenesis causing difficulties in separating biological effects of chemotherapy from those due to angiogenesis inhibitors. This randomized phase II trial was designed to evaluate the additional biomarker effect on angiogenesis when bevacizumab is added to docetaxel.

Experimental Design: Patients with inoperable breast cancer were randomized to either 2 cycles of preoperative docetaxel (D) 35 mg/m² i.v. weekly for 6 weeks, followed by a 2-week break; or docetaxel with bevacizumab 10 mg/kg i.v. every other week for a total of 16 weeks (DB). Plasma and serum markers of endothelial damage, dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), and tumor microvessel density were assessed before treatment and at the end of each preoperative cycle.

Results: Forty-nine patients were randomized (DB, 24; D, 25). There was no difference in overall clinical response, progression-free survival, or overall survival. Vascular endothelial growth factor increased during treatment; more so with DB (P < 0.0001). Vascular cell adhesion molecule-1 (VCAM-1) also increased (P = 0.069). Intercellular adhesion molecule increased (P = 0.018) and E-selectin decreased (P = 0.006) overall. Baseline levels of VCAM-1 and E-selectin correlated with clinical response by univariate analysis. DCE-MRI showed a greater decrease in tumor perfusion calculated by initial area under the curve for the first 90 seconds in DB (P = 0.024). DCE-MRI also showed an overall decrease in tumor volume (P = 0.012).

Conclusion: Bevacizumab plus docetaxel caused a greater increase in vascular endothelial growth factor and VCAM-1, and a greater reduction in tumor perfusion by DCE-MRI compared with docetaxel. Clinical outcomes of inoperable breast cancer were predicted by changes in VCAM-1 and E-selectin.

Numerous studies have shown that therapeutic disruption of nascent vasculature is effective in mediating tumor regression (1). A primary target for antiangiogenic therapy is vascular endothelial growth factor (VEGF), a potent and specific regulator of tumor angiogenesis and endothelial cell survival (2–8). VEGF also induces vascular permeability, which is a critical step in promoting tumor growth, and induces the expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) on endothelial cells (9–11). VCAM-1 is a type-1 transmembrane glycoprotein important for cell adhesion needed for metastasis and is expressed on endothelial cells in response to VEGF stimulation or inflammation (12). VCAM-1 is also present in a soluble form and may function as attractants for endothelial cells (13). Similar to VCAM-1, ICAM-1 is a member of the immunoglobulin-like adhesion molecules and high circulating levels have been associated with a greater propensity...
The purpose of this study was to determine the added biological effect from an angiogenesis inhibitor to a taxane that possesses antiangiogenic properties. Forty-nine previously untreated patients with locally advanced breast cancer were randomized to receive either weekly docetaxel or weekly docetaxel with bevacizumab every other week for a total of 16 weeks. Biological correlates were obtained before treatment, between two cycles of therapy, and at the conclusion of preoperative treatment. There was a statistically significant reduction in tumor perfusion calculated by dynamic contrast-enhanced magnetic resonance imaging with combination chemotherapy and angiogenic inhibitor compared with single-agent chemotherapy. Although there was no significant difference in VCAM-1, intercellular adhesion molecule, or E-selectin levels between the two treatment groups, the clinical outcomes of these patients could be predicted by changes in VCAM-1 and E-selectin. We believe that these important results can be applied to breast cancer patients receiving preoperative treatment to predict disease outcome.

Bevacizumab (rhuMABVEGF, Avastin; Genentech) is a humanized monoclonal antibody with binding specificity for VEGF. Clinical trials investigating the effect of this antibody with or without chemotherapy in the treatment of locally advanced or metastatic breast cancer have been reported with modest clinical results (20-24). This supports the need to document the direct biological consequence of bevacizumab therapy on breast cancer in vivo to differentiate its effects from that of chemotherapy. Wedam et al. (22) examined tumor biopsy specimens in 21 patients with inflammatory and locally advanced breast cancer treated with bevacizumab. An analysis of breast cancer tissue sampled pretherapy and posttherapy revealed a median decrease of 66.7% in phosphorylated VEGFR2 for metastatic dissemination (14, 15). E-selectin is expressed on endothelial membranes as well as secreted, is up-regulated by inflammation, and is associated with the development of metastasis (16). In addition, fluctuations in E-selectin levels may be a more specific marker of breast cancer than other malignancies (17). Expression of these endothelial adhesion molecules seem to correspond with clinical disease status and, thus, can be investigated as a surrogate marker for disease response (18, 19).

Thrombosis occurred in 8.3% of patients receiving bevacizumab (27). These findings raise the question of the added benefit of bevacizumab to the antiangiogenic effects of docetaxel in the treatment of breast cancer. In an attempt to target tumor vasculature and differentiate the antiangiogenic behavior of chemotherapy from that of a targeted antiangiogenesis inhibitor, we designed a phase II randomized trial of weekly docetaxel versus weekly docetaxel combined with bevacizumab in patients with inoperable breast cancer. We hypothesized that the patients receiving combination therapy with docetaxel and bevacizumab would experience a greater effect on angiogenesis exhibited by a greater reduction in tumor microvascular density, more significant alterations in circulating markers of endothelial adhesion, and a larger decrease in tumor perfusion as measured by DCE-MRI than those receiving docetaxel alone.

Patients and Methods

Patients. Men and women age ≥21 y were eligible if they had newly diagnosed, histologically confirmed, inoperable adenocarcinoma of the breast as confirmed by consensus decision of the multidisciplinary Case Comprehensive Cancer Center Tumor Board. Those with stage IV breast cancer were eligible if they presented with a concurrent locally advanced breast cancer. No prior treatment for breast cancer was allowed and measurable disease was required. Other eligibility criteria included the following: Eastern Cooperative Oncology Group performance status of ≤2, normal organ and bone marrow function as described elsewhere (28), and a calculated left ventricular ejection fraction of ≥45%. Patients were excluded from participation if they had New York Heart Association classification III or IV heart disease, central nervous system metastases, major surgery within 28 d of the start of therapy, or a history of thromboses. Full-dose anticoagulation was not permitted. Informed consent was obtained before the patient undergoing any investigational procedure. In 2005, bevacizumab was found to be of therapeutic benefit for metastatic breast cancer (24), and trastuzumab was determined to be efficacious in the treatment of adjuvant disease (29). These findings changed practice patterns and limited subsequent trial accrual, resulting in early study termination after enrollment of 49 of 60 planned patients.

Treatment. Patients were randomized to receive two preoperative 8-wk cycles of either docetaxel (D) alone or the combination of docetaxel and bevacizumab (DB). In both arms, docetaxel was given as weekly 1-h i.v. infusions of 35 mg/m², followed by a 2-wk break (30). Dexamethasone was administered per standard prescribing recommendations to reduce the incidence and severity of hypersensitivity reactions and fluid retention. Cytokine support was not permitted. Bevacizumab (NCT 7048565) was supplied by the Cancer Therapy Evaluation Program/National Cancer Institute and was administered to patients receiving the combination arm (DB) at a dose of 10 mg/kg i.v. every other week throughout the 2 cycles of treatment (8 doses). Bevacizumab was administered immediately before docetaxel. The first dose of bevacizumab was given over 90 min; subsequent infusion times were sequentially reduced to 30 min if no adverse reaction occurred.

Four to 6 wk after the completion of preoperative therapy, patients considered operable underwent definitive surgery consisting of either a modified radical mastectomy or lumpectomy with a level I/II axillary lymph node dissection. Three to 6 wk after surgery, patients who underwent mastectomy received radiation to the chest wall [50.4 Gy with an additional 10-14 Gy boost for those with close/positive margins or inflammatory breast cancer (IBC)]: those who had breast-conserving surgery received radiation therapy to the breast (45-50.4 Gy with a boost of an additional 16-18 Gy). All patients received supracavitary nodal irradiation (45-50.4 Gy prescribed to a depth of 3 cm). Axillary irradiation (45-50.4 Gy) was added in patients who had an inadequate axillary node dissection (<10 lymph nodes removed) or extensive axillary nodal involvement. For patients with gross residual disease in the axilla, a dose

Translational Relevance

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of up to 59.4 Gy was allowed. There was no elective treatment of the internal mammary lymph node chain.

Four weeks after completion of radiation therapy, patients received 4 cycles of standard adjuvant chemotherapy consisting of doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²; AC) given i.v. every 21 d. Cytokine support was permitted. Patients with hormone receptor positive disease subsequently received 5 y of standard dose tamoxifen or anastrozole (if postmenopausal).

**Study evaluations.** Toxicity evaluations were done on each D, DB, and AC treatment day. Disease response was assessed clinically after cycles 1 and 2 (C1 and C2) of preoperative treatment (D or DB), and after completion of AC. Response Evaluation Criteria in Solid Tumors criteria was used to determine disease response and toxicity assessments were determined using the National Cancer Institute Common Toxicity Criteria, version 2.0. Left ventricular ejection fraction was assessed at baseline, at the end of preoperative chemotherapy (after week 16), before AC and at completion of AC.

**Biological correlates.** Plasma levels of angiogenic cytokines (VEGF, basic fibroblast growth factor) and serum markers of endothelial damage (E-selectin, VCAM-1, and ICAM-1) were measured before beginning preoperative treatment (either D or DB), after completing C1 (week 8) and completing C2 (week 17) of preoperative treatment, and at 3, 6 and 12 mo after the completion of radiation therapy. The protocol for collection and measurement of these markers has been described previously (28). All samples were assayed in triplicate. Intra-assay and interassay coefficients of variation for VEGF were 3.3% and 7.4%; ICAM-1, 3.3% and 6.3%; basic fibroblast growth factor, 10.4% and 14.0%; E-selectin, 3.6% and 7.2%; and VCAM-1, 5.9% and 12.4%. The minimum reportable value for VEGF was 6.4 pg/mL.

**Tissue correlates.** Multiple 14-gauge core needle biopsies of the primary breast tumor were obtained for correlative studies before initiating treatment and after C1 (week 8). Additional tumor tissue was obtained at the time of definitive surgery (approximately week 20). Tissue samples were assessed for microvessel density using techniques previously published (31). Three pathologists independently read each slide.

**Imaging correlates: DCE-MRI Tumor Perfusion Assessments.** DCE-MRI was repeated thrice for each subject: before the start of preoperative treatment, after C1, and after C2. Scans were obtained at 1.5T (Siemens Magnetom Symphony) with a bilateral breast-imaging coil. Tumors were located using images from the standard Siemens’ breast exam. DCE-MRI used T1-w 2d FLASH (TR/TE, 43/4 ms; slices, 5; thickness, 8 mm; gap, 2 mm; matrix, 128 × 128). Field of view between 300 to 350 mm was kept constant within subjects. Precontrast T1 estimations used the dual flip angle approach (32): flip angles of 10° and 50° with 5-point averaging. Dynamic acquisitions comprised 180 acquisitions at 4.8-s intervals. A 50-s, 0.1 mg/kg i.v. injection of Gadolinium–diethylenetriaminepentaacetic acid (Magnevist) was started between acquisition 3 and 4. Regions of interest were obtained from baseline-subtracted enhancement images from 70 s postinjection. Desired voxels were identified with a combination of thresholding and interactive boundary drawing, allowing delineation of tumors with complex spatial distributions. Gadolinium–diethylenetriaminepentaacetic acid concentration versus time curves were calculated per our prior methods (33). The accepted measure of tumor perfusion, the initial area under the curve for the first 90 s postcontrast (IAUGC, see ref. 31), along with the efflux rate constant k_e (34), were obtained. Tumor volume was also calculated (number of region of interest voxels × individual voxel volume).

**Statistical methods.** The primary purpose of this randomized phase II trial was to determine the added effects on angiogenesis from the addition of bevacizumab to the effects on angiogenesis caused by docetaxel. The primary objective was to measure the difference in change in biological parameters between the two treatment arms: tumor-associated biomarkers (microvessel density, VEGF, basic fibroblast growth factor, E-selectin, ICAM-1, and VCAM-1) and tumor perfusion as determined by DCE-MRI. These parameters were assessed in two ways: first, parameters were compared as continuous variables between treatment groups by Student’s t test; and second, parameters were compared at different time points by paired t test. The association between baseline biomarkers and clinical response was evaluated by logistic regression in both univariate and multivariate analyses. t test was used to compare the difference of continuous measurements between two groups after checking normality. The association between two factors was examined by χ² test.

Progression-free survival (PFS) was defined as time from first day of treatment to the date of cancer recurrence, progression, or death, and censored at the date of last follow-up for those without disease progression and still alive. Overall survival (OS) was defined as time from the start of treatment to death, and censored at the time of last assessment for survivors. The probability of PFS and OS were estimated by Kaplan-Meier method (35) and the difference between treatment arms was examined by log-rank test. The predictive value of biomarkers on PFS was examined by Cox model (36) after checking proportional hazard assumption in univariate analysis followed by multivariate Cox regression. Temporal pattern of biological parameters was also summarized using scatter plot superimposed with lowess smoother (37). All tests were two sided and a P value of ≤0.05 was considered statistically significant.

**Results**

**Patient characteristics.** Between April 2002 and August 2005, 49 female patients were randomized to either D (n = 25) or DB (n = 24; Table 1). Seventy-eight percent of patients were Caucasian and 22% were African-American. The advanced nature of the study population is evidenced by 43% presenting with stage III disease, 31% with IBC, 12% having stage IV disease (also with inoperable local disease), and 39% having triple negative tumors. Groups were well-balanced in terms of clinical stage at presentation; however, a greater number of patients in the D arm had HER2-positive and hormone receptor-negative disease.

**Toxicity.** No unusual toxicity was evident during AC treatment; therefore, the focus of this report is on the preoperative treatment. Both regimens were well-tolerated (Table 2). There were no episodes of uncontrolled hypertension, proteinuria, or thrombosis. The most notable grade 3/4 toxicities included neutropenia, stomatitis/pharyngitis, and electrolyte abnormalities. The occurrence of grade 3 toxicity with DB was significantly higher compared with D (96% versus 40%; P < 0.001). However, there was no significant difference in grade 4 toxicity between the two treatments (P = 0.667). One patient receiving DB died of complications from a perforated diverticulitis, and another patient on DB experienced an upper gastrointestinal bleed, which was controlled. Delayed wound healing, defined by an inability to start radiation therapy within 6 weeks of surgery, occurred in 8 patients, without a significant difference between treatment arms (DB, 5; D, 3; P = 0.691). Two patients experienced asymptomatic decreases in left ventricular ejection fraction by 17%; 1 patient (DB) during preoperative treatment, and 1 patient (D) from baseline to end of AC (D). There were no episodes of congestive heart failure, or changes in left ventricular ejection fraction to below the institutional upper limit of normal.

**Response to therapy.** Surgical operability after D or DB constituted a clinical response. The overall clinical response rate was 78% with 19 patients in each treatment arm becoming operable (Table 3). Three patients in each treatment arm had a clinical response but remained inoperable. Three patients receiving D and one patient receiving DB developed progressive disease.
One patient experienced a treatment-related death in the DB arm during neoadjuvant chemotherapy. Of the 38 patients who underwent surgery, the median number of pathologically positive axillary lymph nodes was 1 (range, 0-20); 43% of patients had negative axillary lymph node (no difference between treatment groups). Two patients treated with D had residual ductal carcinoma in situ, consistent with a pathologic complete response (38). The overall clinical response rates for the patients with IBC did not differ with respect to treatment assignment: 7 of the 16 patients became operable after protocol treatment, all achieving a partial pathologic response. There was no difference between the 2 groups in either 60-month PFS (D, 47.3%; DB, 37.2%; \( P = 0.939 \)) or OS (D, 57.1%; DB, 57.6%; \( P = 0.583 \); Fig. 1). The median OS has not been reached.

### Biological and tissue correlates.

Plasma VEGF levels rose sharply from baseline to the end of C2 and with DB compared with a minimal increase in plasma VEGF levels with D (\( P < 0.0001 \)). Plasma VEGF levels remained higher with DB than with D at 3 months postradiation therapy (\( P = 0.011 \); Fig. 2A). There was no significant change in basic fibroblast growth factor levels. Overall, there was a significant gradual increase in VCAM-1 levels from pretreatment to the end of C2 (\( P < 0.0001 \)). There was a trend for a greater increase in VCAM-1 levels among patients receiving DB compared with D, which did not reach statistical significance (\( P = 0.069 \)) and completely resolved by 3 months postradiation therapy (\( P = 0.382 \); Fig. 2B). ICAM levels gradually increased (\( P = 0.018 \); Fig. 2C) and E-selectin gradually decreased (\( P = 0.006 \)) from pretreatment to the end of C2 (Fig. 2D); however, there were no significant differences between treatment arms at any time points. Too few patients were available for analysis of biological correlates at 6 and 12 months postradiation therapy. No significant changes were observed in tumor microvessel density assessment (data not shown).

### Tumor volume and perfusion.

Using DCE-MRI, there was a statistically significant decrease in overall IAUC90 after the completion of C1 and C2 (\( P = 0.007 \), \( P < 0.0001 \); respectively; Fig. 3A).}

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>D</th>
<th>DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of enrolled</td>
<td>49</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>Median age (y)</td>
<td>48 (22-79 y)</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Caucasian</td>
<td>38 (78%)</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>African-American</td>
<td>11 (22%)</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Clinical stage IIB</td>
<td>7 (14%)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Clinical stage III</td>
<td>21 (43%)</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Clinical stage IV</td>
<td>6 (12%)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Subset analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBC</td>
<td>15 (31%)</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>HER2+</td>
<td>11 (22%)</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>HR +</td>
<td>23 (47%)</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Triple negative</td>
<td>19 (39%)</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

Abbreviation: HR, hormone receptor (estrogen receptor or progesterone receptor).

### Table 2. Grade 3/4 toxicity: preoperative treatment only

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>DB</td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytopenia</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Infection without neutropenia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anemia</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Nonhematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Elevated transaminases</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Anorexia/fatigue</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Abdominal pain/nausea/vomiting/dehydration</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Eye tearing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sensory neuropathy</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Melena/gastrointestinal bleed</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Perforated viscous†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>23</td>
</tr>
</tbody>
</table>

*Hypophosphatemia, hypouricemia, hyponatremia, and hypokalemia.
†One treatment-related death. No episodes of uncontrolled hypertension or proteinuria.
This reduction was greater among patients receiving DB compared with D ($P = 0.024$). There was also a statistically significant overall decrease in tumor volume in both groups after the completion of C1 and C2 ($P = 0.004$, $P = 0.012$, respectively; Fig. 3B), with no difference between treatment arms.

**Use of clinical and biological correlatives to predict disease response.** On univariate analysis, baseline VCAM-1 and E-selectin levels were significant inverse predictors of clinical response (operability; $P = 0.033$ and $P = 0.035$, respectively). The odds ratio of having a clinical response favored patients without IBC (odds ratio, 0.065; $P = 0.002$). In multivariate logistic regression analysis, only elevated VCAM-1 and presence of IBC showed a trend toward significance in predicting lower clinical response ($P = 0.062$ and 0.066, respectively).

**Discussion**

The National Comprehensive Cancer Network guidelines support the use of preoperative systemic therapy for the treatment of locally advanced or inoperable breast cancer. This approach enables locally advanced breast cancer to become a model for the investigation of novel anticancer agents by providing accessible tumor and blood specimens at various time points during systemic treatment. The goal of using this model is to gain information about biological correlates that correspond to, or predict, a clinical outcome. We applied this principle to 49 patients with inoperable breast cancer to provide biological evidence of inhibition of angiogenesis that may occur due to chemotherapy (docetaxel) or only when a specific antiangiogenic agent (bevacizumab) is combined with chemotherapy (docetaxel). The hypothesis of this investigation was to show that changes in biological correlatives, i.e., tumor blood perfusion as shown by DCE-MRI, tumor microvessel density, and circulating markers of endothelial damage, would correspond with clinical disease response. We also hypothesized that the changes in these parameters would be greater among those patients treated with the combination of an antiangiogenesis inhibitor and chemotherapy compared with chemotherapy alone.

A total of 49 patients were treated on study. All patients presented with locally advanced disease such that there was a low likelihood of obtaining a complete pathologic response with preoperative single agent docetaxel. All patients also received postoperative doxorubicin and cyclophosphamide so as to not compromise their overall clinical outcome. Thirty-seven patients (76%) achieved a clinical response allowing for surgical resection. Those patients were evenly divided between the two treatment groups. Although clinical response has been shown to be an important indicator in phase II trial design investigating targeting agents, this study was not powered to compare differences in clinical outcomes between the two treatment arms: docetaxel versus docetaxel plus bevacizumab (39). In addition, both agents may be targeting the tumor vasculature, thus resulting in an inferior anticancer effect, especially when compared with other taxanes combined with angiogenesis inhibitors (24, 27).

More importantly, this study adds to the understanding of the changes in tumor-associated biomarkers and their correlation with clinical disease response. Byrne and colleagues (12) found that baseline levels of VCAM-1 were higher among patients with breast cancer and corresponded to a poorer prognosis. This phenomenon was also shown in a study of 92 patients with breast cancer. The use of clinical and biological correlatives to predict disease response. On univariate analysis, baseline VCAM-1 and E-selectin levels were significant inverse predictors of clinical response (operability; $P = 0.033$ and $P = 0.035$, respectively). The odds ratio of having a clinical response favored patients without IBC (odds ratio, 0.065; $P = 0.002$). In multivariate logistic regression analysis, only elevated VCAM-1 and presence of IBC showed a trend toward significance in predicting lower clinical response ($P = 0.062$ and 0.066, respectively).

**Table 3. Clinical outcomes**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>D (n = 25)</th>
<th>DB (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical candidate</td>
<td>19 (76%)</td>
<td>19 (79%)</td>
</tr>
<tr>
<td>Breast conserving surgery</td>
<td>7 (37%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Modified radical mastectomy</td>
<td>12 (63%)</td>
<td>18 (95%)</td>
</tr>
<tr>
<td>Inadequate disease response</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Disease progression on therapy</td>
<td>3</td>
<td>1*</td>
</tr>
<tr>
<td>Delayed wound healing postsurgery</td>
<td>3 (16%)</td>
<td>5 (26%)</td>
</tr>
</tbody>
</table>

*Plus one treatment-related death.

3A). This reduction was greater among patients receiving DB compared with D ($P = 0.024$). There was also a statistically significant overall decrease in tumor volume in both groups after the completion of C1 and C2 ($P = 0.004$, $P = 0.012$, respectively; Fig. 3B), with no difference between treatment arms.

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A total of 49 patients were treated on study. All patients presented with locally advanced disease such that there was a low likelihood of obtaining a complete pathologic response with preoperative single agent docetaxel. All patients also received postoperative doxorubicin and cyclophosphamide so as to not compromise their overall clinical outcome. Thirty-seven patients (76%) achieved a clinical response allowing for surgical resection. Those patients were evenly divided between the two treatment groups. Although clinical response has been shown to be an important indicator in phase II trial design investigating targeting agents, this study was not powered to compare differences in clinical outcomes between the two treatment arms: docetaxel versus docetaxel plus bevacizumab (39). In addition, both agents may be targeting the tumor vasculature, thus resulting in an inferior anticancer effect, especially when compared with other taxanes combined with angiogenesis inhibitors (24, 27).

More importantly, this study adds to the understanding of the changes in tumor-associated biomarkers and their correlation with clinical disease response. Byrne and colleagues (12) found that baseline levels of VCAM-1 were higher among patients with breast cancer and corresponded to a poorer prognosis. This phenomenon was also shown in a study of 92 patients with breast cancer. The use of clinical and biological correlatives to predict disease response. On univariate analysis, baseline VCAM-1 and E-selectin levels were significant inverse predictors of clinical response (operability; $P = 0.033$ and $P = 0.035$, respectively). The odds ratio of having a clinical response favored patients without IBC (odds ratio, 0.065; $P = 0.002$). In multivariate logistic regression analysis, only elevated VCAM-1 and presence of IBC showed a trend toward significance in predicting lower clinical response ($P = 0.062$ and 0.066, respectively).
breast cancer versus 31 matched controls (14). These data correspond with our results on univariate and multivariate analysis showing that increased baseline levels of VCAM-1 was associated with a reduction in disease response. Although there was a trend in demonstrating a greater increase in VCAM-1 levels with combination treatment using docetaxel and bevacizumab compared with docetaxel alone, both treatment arms were associated with a significant increase in VCAM-1 levels during active therapy. This may be due to our enrollment of patients with advanced stage as corroborated in other studies, or may reflect a small sample size examined in our study, or it may simply be a tumor response to chemotherapy (40).

Of interest, our institution investigated treatment of IBC using combination doxorubicin and SU5416, a VEGFR2 tyrosine kinase inhibitor, and found no correlation between VCAM-1 expression and clinical outcome, whereas increasing levels of ICAM-1 correlated with event-free survival (33). Other studies involving breast cancer patients have also found a correlation between higher levels of ICAM-1 and a worse prognosis, especially among patients with metastatic disease (41). Our current study did not show this association. One possible explanation is that ICAM-1 may be a more sensitive marker of advanced or aggressive disease, i.e., IBC. Another possibility is that different cancers, e.g., lung and thyroid may be associated with a different spectrum of secreted adhesion molecules (42, 43).

Our current study using docetaxel with or without bevacizumab showed an association between reduction of E-selectin levels and clinical outcome by univariate analysis ($P = 0.035$). These results are supported by prior studies using SU5416 and doxorubicin therapy for IBC, and a study combining bevacizumab and docetaxel in patients with metastatic breast cancer (23, 33).

Based on our results and those of other investigators, VCAM-1 and E-selectin may represent important surrogates for breast cancer response; however, the changes in quantity may not be specific to the administration of targeted angiogenesis.

![Fig. 2. Changes in levels of angiogenic and endothelial markers over time. All figures show a scatter plot of angiogenic and endothelial markers versus time (days on study), superimposed with lowess smoother (locally weighted scatterplot smoothing) by treatment group; D versus DB. Arrow, the end of C2. A, overall, VEGF levels increased with preoperative treatment. There was no difference in VEGF levels pretreatment, but at the end of C2, VEGF levels with DB were significantly larger than VEGF levels with D. B, overall, there was a gradual increase in VCAM-1 levels with treatment. There was no difference in VCAM-1 levels pretreatment, and only marginally different at the end of C2. C, overall, there was an increase in ICAM-1 with treatment. There was no difference in ICAM-1 levels pretreatment or after C2. D, overall, there was a decrease in E-selectin levels with treatment. There was no difference in E-selectin levels pretreatment or at the end of C2.](https://www.aacrjournals.org/doi/10.1158/1078-0432.CCR-08-1459)
inhibitors. Although our study showed higher VCAM-1 levels in the combination bevacizumab and docetaxel arm compared with docetaxel alone, there was no statistical difference in E-selectin or ICAM-1 levels with the addition of the angiogenesis inhibitor. This may be a consequence of the potency of docetaxel as a chemotherapeutic agent with antiangiogenic properties.

We also noted an increase in plasma VEGF initially with combination docetaxel and bevacizumab therapy, but this increase in VEGF subsequently normalized after completion of DB to levels indistinguishable from those observed in the docetaxel alone cohort. This finding may either reflect a compensatory increase in serum VEGF because of sequestration by bevacizumab, or might relate to the fact that the ELISA assay measures both unbound and bevacizumab-bound VEGF, thereby reflecting a longer serum half-life of antibody-bound VEGF (44). Although several studies have shown a correlation between elevated levels of circulating VEGF and an adverse prognosis among breast cancer patients, we did not find this correlation with our current study, nor our prior investigation with SU5416 and doxorubicin for IBC (23, 33, 45, 46).

DCE-MRI showed a greater decrease in IAUC90 with the combination treatment compared with chemotherapy alone at the end of C1 ($P = 0.024$), reflecting a greater decrease in tumor blood perfusion by the addition of bevacizumab to docetaxel. No further significant changes in IAUC90 were observed after C1, which suggests that the optimal benefit of antiangiogenic therapy may occur early in the course of disease treatment in areas of large tumor burden.

The model of using inoperable disease to investigate biological correlates of clinical disease response proved successful, and the results lend support to the results from other studies involving different patient populations. This study additionally shows the safety and relative efficacy of the combination of the angiogenesis inhibitor bevacizumab and docetaxel. Unfortunately, the relatively small size of this study may have been insufficient to detect true differences in biological markers of angiogenesis, and a larger randomized trial may support a greater biological role of angiogenesis inhibitors. In addition, further investigation of biological correlates should continue to provide information on the optimal administration of antiangiogenic therapy and improve patient selection for these specific treatments.

**Disclosure of Potential Conflicts of Interest**

B. Overmoyer, consultant, Sanofi-Aventis. The other authors disclosed no potential conflicts of interest.
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