VEGF as a Marker for Outcome Among Advanced Breast Cancer Patients Receiving anti-VEGF Therapy with Bevacizumab and Vinorelbine Chemotherapy

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Abstract

Background: Anti-vascular endothelial growth factor therapy (VEGF) is an important new treatment modality in oncology. We sought to determine the efficacy and safety of the humanized monoclonal anti-VEGF antibody, bevacizumab, and vinorelbine as treatment for refractory breast cancer and to explore the role of plasma VEGF as a predictor of treatment outcome.

Experimental Design: Eligible patients had received one or two prior chemotherapy regimens for metastatic breast cancer or recurred within 12 months of adjuvant therapy and had measurable disease and adequate end-organ function. Patients received bevacizumab 10 mg/kg every 2 weeks, and vinorelbine each week, until tumor progression or prohibitive toxicity. Plasma VEGF was measured at baseline.

Results: Among 56 women treated on protocol, bevacizumab and vinorelbine yielded a 34% response rate (95% confidence interval, 22-48%) and median time to progression of 5.5 months. Activity was observed regardless of tumor hormone receptor status or type or extent of prior chemotherapy. Side effects included uncomplicated neutropenia, hypertension, naso-congestion/mucositis, and neuropathy, consistent with well-described side effects of the respective agents. Three patients had impaired wound healing following surgical procedures. There were only rare instances of thrombosis or clinically significant proteinuria. Lower levels of baseline VEGF were associated with longer time to progression.

Conclusions: Bevacizumab and vinorelbine are well tolerated and effective as treatment for refractory breast cancer. Plasma VEGF warrants further evaluation as a prognostic marker for treatment outcome in advanced breast cancer patients receiving anti-VEGF therapy.

Angiogenesis is increasingly seen as a target for antineoplastic therapy. Extensive laboratory and clinical data suggest that tumor growth and dissemination are dependent on the development of a neovasculature and that vascular endothelial growth factor (VEGF) is a primary stimulator of such angiogenesis (1, 2). Drugs that neutralize VEGF or inhibit activity of the VEGF receptors are in active development in oncology, and several have shown activity in the treatment of solid tumors including breast, lung, colorectal, and kidney cancers (2, 3).

Bevacizumab is a humanized monoclonal antibody against human VEGF, with a terminal half-life of 18 to 21 days (4). Side effects of bevacizumab are thought related to its vascular-targeting activity and include hypertension, proteinuria, and risks of hemorrhage or thrombosis. Bevacizumab has been explored as monotherapy in a randomized phase II trial among patients with advanced breast cancer (5). Headache was the dose-limiting toxicity at 20 mg/kg every 2 weeks. The recommended dose for future breast cancer trials was 10 mg/kg every 2 weeks. Subsequent trials have examined bevacizumab in combination with chemotherapy for either refractory breast cancer (6) or as first-line treatment for advanced breast cancer (7). At present, there are no tumor characteristics or molecular markers that identify patients who are particularly likely to benefit from bevacizumab-based therapy. Thus, despite the “targeted” nature of bevacizumab therapy, there is a lack of measures that predict antitumor activity. Measurement of VEGF-driven tumor biology is challenging. Fixed tumor specimens obtained at the time of diagnosis may, or may not, accurately reflect VEGF expression in metastatic disease. Circulating VEGF levels may or may not reflect tumor-based VEGF expression or tumor dependence on VEGF. Treatment with bevacizumab renders plasma levels of VEGF undetectable, precluding dynamic assessment of VEGF after treatment. Despite these limitations, measurement of VEGF at baseline...
Translational Relevance

Antiangiogenesis therapy is emerging as important in treatment of advanced breast cancer, and new, effective, and safe regimens are being explored. To date, there are no established biomarkers that identify patients likely to benefit from bevacizumab or other angiogenesis inhibitors. Building on prior work with vinorelbine and antibody-based therapy for advanced breast cancer, we developed a phase II study to determine the feasibility and activity of vinorelbine and bevacizumab in treatment of advanced breast cancer. We show that this regimen is very well tolerated and has significant response activity. The regimen has moved forward and is being used in randomized trials to define further the role of bevacizumab in advanced breast cancer. We also explored baseline serum VEGF levels as a predictor of outcome. We show that patients with low serum VEGF levels had longer time to tumor progression than those with high levels. This marker, if confirmed by other investigators, could be useful in selecting patients likely to benefit from bevacizumab-based chemotherapy for advanced breast cancer.

Patients and Methods

Eligibility. Eligible patients had stage IV breast cancer with measurable disease by Response Evaluation Criteria in Solid Tumors. Patients had received one or two prior chemotherapy regimens for advanced breast cancer or recurred within 12 months of adjuvant chemotherapy. Patients with HER-2-positive tumors were required to have previous treatment with trastuzumab. Patients could not have received prior vinorelbine or anti-VEGF therapy. Patients could have received prior radiation, endocrine therapy, or trastuzumab therapy provided such therapy concluded before protocol treatment. Patients were required to be ages ≥18 years. Eastern Cooperative Oncology Group performance status 0 to 2, absolute neutrophil count ≥1,500/mm³, hemoglobin ≥9 g/dL, platelets ≥100,000/mm³, bilirubin ≤1.5 times institutional upper limit of normal, aspartate aminotransferase/alanine aminotransferase ≤1.5 times institutional upper limit of normal, creatinine <2 mg/dL, left ventricular ejection fraction ≥50%, and urine protein either 1+ or less on dipstick measurement or <500 mg protein/24 h.

Patients with brain metastases, bleeding or clotting diatheses, requirement for systemic anticoagulation, chronic aspirin therapy in excess of 325 mg/d or nonsteroidal anti-inflammatory drug therapy, or international normalized ratio >1.5 were ineligible. Patients with recent (28 days) major surgical procedure, open biopsy, or significant traumatic injury, or anticipation of need for major surgical procedure during the course of the study, were also ineligible. All patients provided written informed consent.

The study was conducted in cooperation with the Cancer Therapy Evaluation Program/National Cancer Institute (protocol 2716) and approved by the institutional review boards of Dana-Farber/Harvard Cancer Center. Accrual was from March 2001 to September 2002.

Treatment Plan. Patients received bevacizumab 10 mg/kg every other week, supplied as an investigational agent by the National Cancer Institute Division of Cancer Treatment and Diagnosis under a Cooperative Research and Development Agreement or Clinical Trials Agreement between Genentech and the National Cancer Institute Division of Cancer Treatment and Diagnosis. Urine protein was monitored every 8 weeks, and patients who developed new proteinuria or worsening proteinuria were evaluated with 24 h urine collection. Those with proteinuria ≥2,000 mg/24 h were removed from study. Patients received commercially available vinorelbine every week at a dose of 25 mg/m². As in our previous experience with vinorelbine (11), dose was adjusted to 15 mg/m² for absolute neutrophil count 750 to 1,250/mm³ or neutropathy in excess of grade 1 and to 12.5 mg/m² for bilirubin 2 to 3 mg/dL as measured on each treatment day. Patients could receive antiemetics, growth factors, and other supportive care according to standard institutional practice. Vital signs were measured every 2 weeks. Patients who developed hypertension (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg) were advised to initiate or add antihypertensive therapy with an angiotensin-converting enzyme inhibitor followed subsequently with use of either diuretic or β-blockers. Patients with grade 4 hypertension or persistent hypertension despite optimal drug therapy were taken off study. Patients experiencing most other treatment-related nonhematologic toxicity in excess of grade 2 had therapy held until resolution of symptoms to grade ≤1.

VEGF Testing. Plasma VEGF levels were measured at baseline using the following methods (12). Microwell plates were coated with two murine monoclonal antibodies (Mab1554 and Mab3305) at 0.5 μg/mL each and incubated overnight at 2°C to 8°C. Diluted human plasma samples, standards, and controls were added and incubated at 37°C. Subsequently, biotin-conjugated Mab3305 was added and incubated followed by streptavidin conjugated to β-galactosidase. Diluted 4-methylumbelliferyl-β-D-galactopyranoside substrate was added and incubated overnight at 37°C in the dark. The substrate reaction was stopped with 150 mmol/L glycine. The plates were read using a fluorescence reader with a 360 nm filter for excitation and a 460 nm filter for emission. The standard curve was recombinant human VEGF165/165 diluted in sample diluent from 1 to 128 pg/mL with a reporting range of 12.5 to 445 pg/mL human plasma.

Analytical Plan. The primary study endpoint was response rate; secondary endpoints included characterization of time to progression (TTP), treatment safety, and exploratory analyses of predictors of response/TTP. The study had a two-stage design; with at least 6 responses among the first 19 patients, another 18 patients would be entered on study. We observed 11 responses among the first 22 evaluable patients and elected to expand accrual from 37 to 56 patients to better define clinical activity of the regimen. The original
design called for 18 patients to be entered on the second stage of accrual, with a decision rule based on a null hypothesis response rate of 25% and an alternative hypothesis response rate of 45%. The total accrual of 56 allowed the study to test a null hypothesis of 30%. The chemotherapy combination would be deemed worthy of further study if at least 22 responses were seen out of 56 patients. With this design, there was an 8% chance of deciding the combination is worthy of further study if the true response rate was 30% and an 80% chance of deciding the combination is worthy of further study if the true response rate was 45%. Fisher’s exact test and a step-up logistic regression were used to assess the effect of patient characteristics on response percent. For the analyses of TTP, defined from time of registration, the Kaplan-Meier curve estimate and a step-up Cox proportional hazards regression model were used. For both models, variable selection was based on the likelihood ratio test. The relationship of baseline VEGF level to the other covariates in this study was explored using the Wilcoxon rank-sum test and the Kruskal-Wallis test.

Table 1. Clinical characteristics, tumor response, and TTP

<table>
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<th>Factor</th>
<th>n</th>
<th>Overall response percent</th>
<th>P (Fisher’s exact)</th>
<th>Median TTP (mo)</th>
<th>P (log-rank)</th>
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<td>41</td>
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Results

Clinical efficacy. Fifty-six women (median age, 52.6 years; range, 30-76) entered this phase II study. Seven patients later found ineligible by virtue of the timing or extent of prior therapy were included in the treatment and correlative analyses. The patient population included women heavily pretreated with chemotherapy (Table 1). Most patients (86%) had received adjuvant chemotherapy. In the metastatic setting, patients had received 0 (21%), 1 (48%), or 2 (30%) lines of chemotherapy. The fraction of patients who had received specific types of chemotherapy is shown in Table 1; 57% of patients had received both anthracyclines and taxanes. Sites of metastatic disease included lung (54%), liver (54%), bone (46%), lymph node (52%), soft tissue (11%), and chest wall/breast (14%); 82% of patients had visceral metastases. Nearly half the patients had more than two sites of metastatic disease. Fifty-nine percent of tumors were hormone receptor positive [52% being estrogen receptor (ER) positive]. Forty-two patients came off study for progressive disease, 4 for treatment-associated toxicity, 1 because of death, and 9 for other reasons.

The overall response rate (complete plus partial responses) was 34% (95% confidence interval, 22-48%; Table 1). The regimen was active in patients with extensive prior treatment, including a response rate of 29% as third-line chemotherapy for metastatic disease, and in excess of 30% among patients who had received prior anthracycline or taxane therapy. Neither age, hormone receptor status, or the type or extent of prior chemotherapy or endocrine therapy was an independent predictor of tumor response or TTP. In the step-up logistic models for response, having more than one organ site with disease was the only significant covariate \( (P = 0.002) \); for unclear reasons, patients with multiple sites of disease were more likely to respond to treatment. Response rates tended to be higher among ER-negative tumors (44% versus 24%; \( P = 0.16 \)), although median TTP was 6.0 months in ER-positive cases and 4.7 months in ER-negative cases.

Median TTP was 5.5 months (Table 1; Fig. 1). Actuarial analysis suggested that 20% of patients were progression free at 1 year. Neither age, tumor hormone receptor status, prior endocrine therapy, adjuvant chemotherapy, or line of chemotherapy emerged as significant predictors of TTP.

Baseline VEGF as a marker of outcome. Plasma VEGF was measured at baseline, and results were available for 49 of 56 patients. Figure 2 shows the distribution of baseline VEGF levels expressed as pg/mL. Seven patients had baseline VEGF levels <12.5 pg/mL, the minimum detectable level, and 2 patients had VEGF levels >445 pg/mL, the maximum detectable level. The overall median of VEGF level was 32.6 pg/mL. Wilcoxon rank-sum test and Kruskal-Wallis test were used to evaluate the association between VEGF level and patient characteristics listed in Table 1. No significant associations were found. However, the VEGF levels tended to be higher in patients with ER-positive status, age >50 years, bone metastases, or more than one disease site and lower in patients with liver metastases or with prior chemotherapy for metastatic disease.

To better describe the associations between VEGF level and response rate as well as TTP, we categorized VEGF level into three categories around the median: unknown, 0 to 32.6 pg/mL, or >32.6 pg/mL. There was no association between VEGF level and response. However, there appeared to be a relationship between VEGF level and TTP. A step-up Cox proportional hazards model identified VEGF level >32.6 as a significant variable \( (P = 0.003) \) after adjusting for VEGF unknown), whereas the number of disease site greater than one was marginally significant \( (P = 0.08) \). Figure 3 shows TTP by three VEGF categories (unknown, ≤32.6, and >32.6). Median TTP was 3.7 months for patients with VEGF >32.6 pg/mL, 9.3 months for patients with VEGF ≤32.6 pg/mL, and 7.9 months for patients with unknown VEGF levels.

Toxicity. Treatment with bevacizumab and vinorelbine was well tolerated (Table 2). Grade 4 toxicities were uncommon and limited to neutropenia seen in 30% of patients. Consistent with prior descriptions of bevacizumab activity, 16% of patients developed grade 3 hypertension, requiring initiation or addition of antihypertensive therapy. Clinically significant bleeding was rare; a substantial fraction of patients developed nasal congestion and associated minor degrees of epistaxis.

![Fig. 1. TTP for patients on study with 95% confidence intervals. Median TTP = 5.5 mo.](image1)

![Fig. 2. Distribution of baseline plasma VEGF levels in patients with refractory breast cancer (in pg/mL).](image2)
Ten patients were given granulocyte colony-stimulating factor. Expression, and limited degrees of gastrointestinal discomfort. chemotherapy, including peripheral neuropathy, myelosup-
side effects were typical of those seen with vinorelbine-based
of bacterial superinfection and despite antibiotic therapy. Other
persistent open wounds lasted for weeks without overt evidence
device and removal of a breast implant. In these cases, procedures, including removal of an indwelling venous access
disability following surgical
embolism. There was a single case of grade 3 proteinuria. Three
patients had impaired wound healing following surgical
experiencing side effects familiar to use of each agent. Novel
elbine and bevacizumab was well tolerated, with patients favorably with these previous results. Treatment with vinor-
in this study population, yielding clinical responses in about
one-third of patients despite the previous chemotherapy. The median TTP of 5.5 months. The historic experience with
capecitabine was compared with capecitabine alone (6). That
report did not suggest a significant improvement in TTP with
of advanced ovarian cancer has been shown to lower serum
chemotherapy when paired with bevacizumab in the treatment
associated with greater likelihood of tumor response, without
cancer, higher levels of serum VEGF at baseline were
patients with one or two prior chemotherapy regimens
bevacizumab treatment but did not identify selectively which
tumors might benefit from therapy (14). Our study sought to
characterize patterns of plasma VEGF levels in women with
advanced breast cancer and to determine whether baseline
plasma VEGF levels might be associated with treatment
outcome in breast cancer. We found a broad distribution of
baseline VEGF. In addition, we found that lower circulating
levels were associated with longer TTP, whereas higher VEGF
levels were associated with shorter TTP and no instances of
extended tumor control. Because this was a single-arm study, it
is not possible to distinguish VEGF as a prognostic or a
predictive marker for bevacizumab-based therapy. However,
these findings are consistent with reports from treatment of
advanced colorectal cancer, where higher baseline levels of
VEGF were associated with a shorter disease-free and overall
survival in response to chemotherapy with or without
bevacizumab, although VEGF levels were not predictive of
benefit from bevacizumab therapy (12). It is unclear whether
either systemic or peritumoral VEGF levels are significant
predictors of bevacizumab activity. Because VEGF is only one of
many mediators of angiogenesis, its significance as a prognostic
marker in antiangiogenic treatment could be argued several
different ways. Tumors with high levels might be especially
could be targets; in contrast, such tumors might elaborate
multiple angiogenic factors, rendering neutralization of VEGF
less critical. In primary, lymph node-negative breast cancer,
intratumoral levels of VEGF have proven to be an adverse
prognostic marker (15).

Other trials have reached different conclusions regarding
baseline VEGF levels and bevacizumab-associated outcomes. In
a trial of bevacizumab paired with docetaxel for advanced
breast cancer, higher levels of serum VEGF at baseline were
associated with greater likelihood of tumor response, without
comment on the effect on TTP (16). Low-dose, metronomic
chemotherapy when paired with bevacizumab in the treatment
of advanced ovarian cancer has been shown to lower serum
VEGF levels without correlation with clinical outcomes such as
response or progression (17). Finally, we acknowledge that
neither techniques for measuring VEGF nor cutoff levels for
analyzing VEGF and clinical outcomes are standardized.
Clearly, additional work is needed to determine the role of
VEGF as a prognostic marker for bevacizumab-based therapy.

The role for bevacizumab in advanced breast cancer is
evolving. Randomized trials have yielded mixed results. In a
study of patients with one or two prior chemotherapy regimens
for advanced breast cancer or recurrence within 12 months of
adjuvant chemotherapy, the combination of bevacizumab with
capcitabine was compared with capcitabine alone (6). That
report did not suggest a significant improvement in TTP with
the addition of bevacizumab (4.9 versus 4.2 months), although
there was an improvement in investigator-reported response
rate (30% versus 19%). The reported TTP and response rate in
our study of vinorelbine plus bevacizumab, among a similar
patient population, show generally comparable rates of TTP
and disease as the capcitabine and bevacizumab combin-
In contrast to results seen in treatment of more advanced
disease, a randomized trial of first-line chemotherapy with
paclitaxel alone or paclitaxel in combination with bevacizumab

(21%) with treatment; one patient had grade 3 epistaxis. There
was a single case of grade 3 thrombocytopenia, a pulmonary
embolism. There was a single case of grade 3 proteinuria. Three
patients had impaired wound healing following surgical
procedures, including removal of an indwelling venous access
device and removal of a breast implant. In these cases,
per Persistent open wounds lasted for weeks without overt evidence
bacterial superinfection and despite antibiotic therapy. Other
side effects were typical of those seen with vinorelbine-based
chemotherapy, including peripheral neuropathy, myelosup-
pression, and limited degrees of gastrointestinal discomfort.

Discussion

Antiangiogenic therapies are emerging as important in breast
cancer, but neither the optimal use of such treatments nor
predictors of outcome or benefit are clear. Building on our prior
experience combining vinorelbine chemotherapy with human-
ized monoclonal antibody treatments, we paired vinorelbine
and bevacizumab to determine the efficacy of this combination
in refractory breast cancer. Vinorelbine plus bevacizumab
showed substantial activity despite the extent of prior treatment
in this study population, yielding clinical responses in about
one-third of patients despite the previous chemotherapy. The
median TTP of 5.5 months. The historic experience with
vinorelbine in treatment-refractory breast cancer suggests
response rates on the order of 15% to 25%, with TTP between
3 and 6 months (13); we believe our outcomes compare
favorably with these previous results. Treatment with vinor-
elbine and bevacizumab was well tolerated, with patients
experiencing side effects familiar to use of each agent. Novel
side effects associated with bevacizumab therapy included
hypertension and nasal congestion/epistaxis and, in some
instances, concerns over wound healing.

Although the target of bevacizumab, VEGF, is well estab-
lished, there are at present no known predictors of benefit from
bevacizumab therapy. Experience in the neoadjuvant treatment
of breast cancer using chemotherapy and bevacizumab
identified changes in VEGFR expression and apoptosis during
bevacizumab treatment but did not identify selectively which
tumors might benefit from therapy (14). Our study sought to
characterize patterns of plasma VEGF levels in women with
advanced breast cancer and to determine whether baseline
plasma VEGF levels might be associated with treatment
outcome in breast cancer. We found a broad distribution of
baseline VEGF. In addition, we found that lower circulating
levels were associated with longer TTP, whereas higher VEGF
levels were associated with shorter TTP and no instances of
extended tumor control. Because this was a single-arm study, it
is not possible to distinguish VEGF as a prognostic or a
predictive marker for bevacizumab-based therapy. However,
these findings are consistent with reports from treatment of
advanced colorectal cancer, where higher baseline levels of
VEGF were associated with a shorter disease-free and overall
survival in response to chemotherapy with or without
bevacizumab, although VEGF levels were not predictive of
benefit from bevacizumab therapy (12). It is unclear whether
either systemic or peritumoral VEGF levels are significant
predictors of bevacizumab activity. Because VEGF is only one of
many mediators of angiogenesis, its significance as a prognostic
marker in antiangiogenic treatment could be argued several
different ways. Tumors with high levels might be especially
could be targets; in contrast, such tumors might elaborate
multiple angiogenic factors, rendering neutralization of VEGF
less critical. In primary, lymph node-negative breast cancer,
intratumoral levels of VEGF have proven to be an adverse
prognostic marker (15).

Other trials have reached different conclusions regarding
baseline VEGF levels and bevacizumab-associated outcomes. In
a trial of bevacizumab paired with docetaxel for advanced
breast cancer, higher levels of serum VEGF at baseline were
associated with greater likelihood of tumor response, without
comment on the effect on TTP (16). Low-dose, metronomic
chemotherapy when paired with bevacizumab in the treatment
of advanced ovarian cancer has been shown to lower serum
VEGF levels without correlation with clinical outcomes such as
response or progression (17). Finally, we acknowledge that
neither techniques for measuring VEGF nor cutoff levels for
analyzing VEGF and clinical outcomes are standardized.
Clearly, additional work is needed to determine the role of
VEGF as a prognostic marker for bevacizumab-based therapy.

The role for bevacizumab in advanced breast cancer is
evolving. Randomized trials have yielded mixed results. In a
study of patients with one or two prior chemotherapy regimens
for advanced breast cancer or recurrence within 12 months of
adjuvant chemotherapy, the combination of bevacizumab with
capcitabine was compared with capcitabine alone (6). That
report did not suggest a significant improvement in TTP with
the addition of bevacizumab (4.9 versus 4.2 months), although
there was an improvement in investigator-reported response
rate (30% versus 19%). The reported TTP and response rate in
our study of vinorelbine plus bevacizumab, among a similar
patient population, show generally comparable rates of TTP
and disease as the capcitabine and bevacizumab combin-
In contrast to results seen in treatment of more advanced
disease, a randomized trial of first-line chemotherapy with
paclitaxel alone or paclitaxel in combination with bevacizumab

![Fig. 3. TTP as a function of baseline plasma VEGF levels for patients with VEGF < 32.6 (line 1), VEGF > 32.6 (line 2), or unknown (line 3).](image)
reported a significant improvement in TTP (11 versus 6 months) and response (30% versus 14%) for the addition of bevacizumab therapy. It is not clear what accounts for the seemingly different effect of bevacizumab in the different treatment settings. In particular, it is not known if aspects of tumor evolution during treatment contribute to resistance to chemotherapy/bevacizumab treatment or whether the chemotherapy backbone of treatment is an important determinant of outcome. A single-arm, phase II study of bevacizumab plus capecitabine as first-line treatment for advanced breast cancer identified a response rate of 38% and median TTP of 5.7 months (18). Comparison of regimen activity across phase II or III studies is fraught with difficulty. Nonetheless, we believe that the results with vinorelbine plus bevacizumab suggest at least comparable efficacy to other bevacizumab-based chemotherapy regimens in advanced breast cancer. These results are the first to analyze VEGF levels as a predictor of outcome in advanced breast cancer patients receiving bevacizumab therapy. Our findings suggest that plasma VEGF could serve as a prognostic marker for tumor progression among such patients. We believe these are provocative observations worthy of study in other clinical trials.

Disclosure of Potential Conflicts of Interest

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