Breast Cancer: Targets and Therapy

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REVIEW

Evidence for the role of bevacizumab in the treatment of advanced metastatic breast cancer: a review

This article was published in the following Dove Press journal:
Breast Cancer: Targets and Therapy
18 June 2010
Number of times this article has been viewed

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Abstract: Angiogenesis inhibitors may provide a new approach to the treatment of metastatic breast cancer. Bevacizumab is a monoclonal antibody against pathologic angiogenesis. A pivotal study (ECOG 2100) showed that bevacizumab in combination with paclitaxel increased progression-free survival for patients with metastatic breast cancer by 6 months. Subsequently, several clinical trials have shown that the combination of bevacizumab with a taxane can improve disease-free survival but does not prolong overall survival. While generally well tolerated, bevacizumab is potentially toxic for some patients who develop hypertension, proteinuria, bleeding, impaired wound healing, bowel perforation or thromboembolic events. Here, we review the current evidence for the use of bevacizumab in breast cancer and ongoing studies that address the questions of how to optimize regimens and schedules for the use of anti-angiogenic agents and the identification of those patients who would benefit the most from treatment with regimens that include antiangiogenic therapy.

Keywords: bevacizumab, chemotherapy, angiogenesis inhibitors, breast cancer

Introduction

The umbilical vessels join on the uterus like the roots of plants and through them the embryo receives its nourishment.
– Aristotle, *On the Generation of Animals*, ca. 340 B.C.

Breast cancer remains the most common cancer among women and the second leading cause of cancer deaths in women. Globally, there are an estimated 4.4 million women alive who have been diagnosed with breast cancer within the last 5 years. Approximately 30% of women with earlier stages of breast cancer will eventually progress to metastatic disease.[1] Women with aggressive basal subtype or triple negative cancers (negative estrogen receptors, negative progesterone receptors and HER2 negative) are at particularly high risk of developing metastases even when they present at early stages.[2,3] As large numbers of women are living with metastatic breast cancer (MBC) for longer periods of time, the need for clinical trials programs and services for this population is becoming increasingly important. Despite advances in breast cancer therapeutics and drug development, it remains difficult to adequately predict whether patients will even respond at all to treatment and which MBC patients will respond to specific regimens. Angiogenesis inhibitors provide new possibilities for the treatment of women.

Angiogenesis (from the Greek angeion meaning vase and genesis meaning birth) is defined as the outgrowth of new blood vessels from pre-existing vessels. The term angiogenesis was first used in the 1700s by John Hunter to describe blood vessel

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growth in reindeer antlers in response to cold exposure.[4] Angiogenesis was later used to describe the formation of new vessels in the placenta and developing embryo of monkeys by Arthur Hertig in 1935.[5] In the 1940s, Algire and Chalkeley at the National Cancer Institute observed that blood vessels migrate toward tumors in wound chambers, demonstrating that tumors actively attract new blood vessels.[6]

Dr Judah Folkman at Harvard Medical School was the first to postulate that the process of tumor angiogenesis could potentially be targeted for cancer treatment.[7] The first angiogenesis inhibitor was identified in cartilage by Brem and Folkman in 1971.[8] Using the rabbit cornea as an assay, they demonstrated that a cartilage implant decreased the rate of capillary growth, induced by tumor, by an average of 75%.

In 1989, Napoleone Ferrara and Jean Plouet discovered a growth factor for vascular endothelial cells in conditioned media they named vascular endothelial growth factor (VEGF).[9] VEGF was later demonstrated to be identical to a factor in tumor ascites that rapidly increased microvascular permeability, identified by Dr Harold Dvorak in 1983 as vascular permeability factor (VPF).[10]

Elevated levels of VEGF were observed in solid tumors and correlated with worse clinical outcomes. Ultimately, angiogenesis became recognized as an essential step in tumor growth and metastasis.[11] Antiangiogenesis has provided exciting possibilities for cancer treatment and in 2004 the United States Food and Drug Administration (FDA) Commissioner Mark McClellan pronounced antiangiogenic therapy "the fourth modality for cancer treatment", establishing that this therapy was potentially as important as surgery, chemotherapy and radiation in the treatment of cancer.

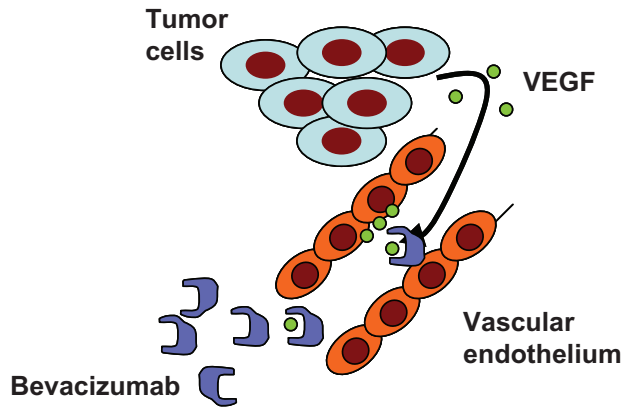

Figure 1 Tumor cells secrete various isoforms of VEGF in a paracrine fashion. VEGFs stimulate the growth, division and permeability of vascular endothelial cells through interaction with cellular VEGF-receptors (VEGFR1 and VEGFR2). This process of neovascularization can be disrupted by the neutralizing humanized monoclonal antibody bevacizumab.

Bevacizumab (Avastin®) was developed as a monoclonal antibody against all isoforms of VEGF-A, which is the isoform responsible for pathologic angiogenesis.[12,13] The original mouse monoclonal antibody that was most effective in neutralizing VEGF was then humanized using recombinant technology (Figure 1).

The efficacy of bevacizumab has been shown in colorectal cancer,[14] non-small-cell lung cancer (NSCLC)[15,16] renal cancer and ovarian cancer.[17,18] Clinically, bevacizumab has been investigated in combination with a range of chemotherapeutic agents, and the pharmacokinetics and toxicities have generally been non-overlapping. Bevacizumab is currently approved in the US for the treatment of NSCLC in combination with paclitaxel and carboplatin, based on the outcome of ECOG (Eastern Cooperative Oncology Group) E4599.[16] Bevacizumab is also approved for the first-line treatment of patients with MBC in combination with paclitaxel, based on ECOG 2100;[19] and in metastatic colorectal cancer in combination with irinotecan and 5-fluorouracil/leucovorin.[20] In addition to metastatic lung and colorectal cancer, bevacizumab was recently also approved for the treatment of metastatic renal cell cancer, based on the results of the Avastin + Interferon vs Placebo + Interferon (AVOREN) trial. The AVOREN study showed an increase in progression-free survival (PFS) for renal cancer patients who received bevacizumab plus interferon alpha, compared to patients who were treated with interferon alpha alone.[21] Additionally, the FDA granted approval for the use of single agent bevacizumab for the treatment of recurrent glioblastoma multiforme, based on two single arm studies.[22,23] While the approvals in lung and colorectal cancer were based on an improved overall survival with bevacizumab,[16,20] the approvals in metastatic renal and breast cancer as well as in glioblastoma were based on an improvement in progression-free survival.

Bevacizumab is being studied worldwide in more than 300 clinical trials and in more than 20 different tumor types, leading to high expectations for its potential to help conquer breast cancer as well.

Pharmacology and pharmacokinetics

The crucial step in neo-vascularization is the "angiogenic switch",[24] which allows tumors to usurp the growth mechanisms of normal vascular endothelial cells and develop to macroscopic size. This process is mediated by the interaction of VEGFs with their membrane-bound receptors (VEGF-Rs).

To date, there are 6 known VEGFs (A, B, C, D, E and placental growth factor) that bind and activate 3 different endothelial cell-bound receptor-tyrosine kinases (VEGFR-1, -2 and -3). Best examined in the context of tumor angiogenesis is VEGF-A, which is expressed in 6 differentially spliced isoforms that bind both VEGFR-1 (flt) and VEGFR-2 (flk), and that stimulate the proliferation of endothelial cells as well as increase the permeability of vasculature.[25,26]

Upregulation of VEGF expression is a hallmark of many tumors, including invasive ductal carcinomas of the breast,[27,28] and has been closely linked to expression of other growth factors such as TNF-α, EGF, insulin, insulin-like growth factor 1 and estrogen.[12,29] *In vitro* VEGF secretion is increased upon loss of the tumor suppressor p53 or amplification of oncogenes such as HER2.[30,31]

Therapeutic disruption of tumor neo-vascularization has been achieved in two ways. Firstly, the VEGFs can be neutralized by using bevacizumab, which recognizes all isoforms of human VEGF,[32] thereby eliminating the ligands required for VEGFR activation and the mitogenic and permeability-enhancing stimuli necessary for neo-vascularization. Secondly, the signal transduction cascade downstream from VEGFRs can be disrupted by using the small molecule inhibitors sorafenib and sunitinib.[32]

Given that bevacizumab is a monoclonal antibody, it is distributed to highly perfused areas with a linear kinetic profile. The terminal elimination half-life of bevacizumab is measured in weeks.[33] Currently, recommended dosing is 10 mg/kg every 2 weeks, for complete suppression of serum VEGF.[34,35]

Efficacy in clinical trials

One of the first trials of bevacizumab in breast cancer was a phase 1 and 2 trial of 75 patients with previously treated metastatic breast cancer.[36] In this study bevacizumab monotherapy resulted in an overall response rate of 9.3%; 17% of patients had a response or were stable at 22 weeks. The treatment toxicity of bevacizumab monotherapy was low and differed from toxicity profiles of classical cytotoxic therapies, which lent support for subsequent trials in metastatic breast cancer combining bevacizumab with chemotherapy.[36]

Given these findings, a phase 3 trial to look at the addition of bevacizumab to capecitabine (Xeloda®) was undertaken, also in pretreated breast cancer patients.[37] The combination of bevacizumab with capecitabine in patients with previously treated metastatic cancer demonstrated a significant increase in the response rate from 9.1% to 19.8%, but PFS and overall survival did not improve.[37] Unfortunately, the improvements in response appeared to be short-lived.

Following this, two major trials, the ECOG 2100 and the Avastin and Docetaxel (AVADO) trials were designed to look at the addition of bevacizumab to a taxane. The ECOG trial 2100 employed paclitaxel and the AVADO trial docetaxel. The truly pivotal study for metastatic breast cancer was ECOG 2100.[19] ECOG 2100 compared paclitaxel alone with paclitaxel plus bevacizumab as initial treatment in a multi-institutional randomized phase 3 trial of 722 patients with metastatic breast cancer. The paclitaxel was given weekly, with biweekly bevacizumab at 10 mg/kg dosing. Results showed that the median progression free survival (PFS) was increased from 6.7 to 13.3 months with the addition of bevacizumab, resulting in a 52% reduction in the risk of disease progression ($P < 0.0001$). The study was stopped early, following a recommendation of the Independent Data Monitoring Committee. However, despite the improvement in disease-free survival, bevacizumab did not prolong overall survival.[38]

Simultaneously, the AVADO study investigated the efficacy of bevacizumab in addition to standard first-line treatment of metastatic breast cancer with docetaxel. This study has a 3-arm design, with all patients receiving docetaxel at 100 mg/m^2, in combination with either placebo, bevacizumab at 7.5 mg/kg or 15 mg/kg respectively every 3 weeks. A total of 736 patients with metastatic breast cancer were enrolled internationally, and the findings were presented at a median follow-up of 11 months at the 2008 ASCO meeting.[39] The study showed that the median time to disease progression was 8 months with docetaxel alone, 8.7 months with docetaxel plus low-dose bevacizumab, and 8.8 months with docetaxel plus high-dose bevacizumab. Thus, while the addition of bevacizumab to docetaxel did not appear to add significantly to the treatment toxicity, the magnitude of the benefit that bevacizumab added to treatment with docetaxel appeared to be much lower then the benefit observed in combination with weekly paclitaxel (ECOG 2100). After a median follow-up of 11 months, the AVADO trial showed a statistically significant difference in overall response rate and PFS overall; the high dose had better efficacy than the low dose.[39] Mature data after a median follow up of 25 months confirmed the improvement in PFS and overall response rate, but no difference in overall survival.[40]

Based on promising results of ECOG 2100 and the AVADO studies, both of which showed improvements in progression free survival, the FDA approved bevacizumab, in combination with paclitaxel, as a treatment of HER2-negative

metastatic breast cancer in February 2008. Bevacizumab was approved for patients with advanced breast cancer under the FDA's accelerated approval program, which allows the FDA to approve products for cancer or other life-threatening diseases based on initial positive clinical data. This decision was somewhat unexpected as a previous FDA advisory committee had recommended against approval.

The FDA approval allowed the next set of trials to proceed. The Avastin + Chemotherapy vs Placebo + Chemotherapy (RiBBON) trials are large multicenter, randomized phase 3 studies that are being conducted to examine the impact of bevacizumab in combination with standard first line chemotherapy regimens for metastatic breast cancer.[41,42] The RiBBON 1 trial enrolled patients with previously untreated metastatic breast cancer while the RiBBON 2 trial enrolled those with previously treated metastatic disease.

In the RiBBON 1 trial (AVF3694 g) patients were randomized to receive bevacizumab plus chemotherapy or placebo plus chemotherapy. RiBBON-1 is a global, double-blind, randomized phase III trial with 1,237 patients in 22 countries who did not receive previous chemotherapy for their HER2-negative metastatic breast cancer. RiBBON-1 evaluated bevacizumab with different types of chemotherapies in patients with advanced HER2-negative breast cancer. One group received capecitabine in combination with bevacizumab, the other taxane- or anthracycline-based chemotherapy. Bevacizumab was administered at 15 mg/kg dose every 3 weeks. The median follow-up was 15.6 months in the cohort that received capecitabine and 19.2 months in the cohort that received a taxane and anthracycline. This resulted in a statistically significant improvement in progression free survival. While there was a modest increase in progression-free survival (from 6.2 to 9.8 months in the capecitabine arm, and from 8.3 to 10.7 months in the taxane/anthracycline arm), there was again no significant benefit for overall survival in the RiBBON study, as reported at the 2009 ASCO meeting.[41,42] A full review of both the AVADO and RiBBON I data by the FDA will be required for the accelerated approval to be converted into a full approval.

The investigation of the best use of bevacizumab is ongoing. Given its efficacy in combination with chemotherapy, and its likely function as an enhancer of the treatment efficacy rather than an independent agent, bevacizumab is currently under investigation in combination with a range of chemotherapeutic agents. Current trials are being conducted to examine the impact of bevacizumab in combination with standard first line chemotherapy regimens for metastatic breast cancer. The most promising combination, paclitaxel in combination with bevacizumab, is currently being investigated in the adjuvant setting (ECOG 5103) for patients with early-stage HER2-negative breast cancer. This phase 3 trial began enrollment in November 2007 and 3,487 of the planned 4,950 patients have been enrolled to date.

A second area of intense interest is whether bevacizumab increases the activity of targeted agents in breast cancer. Combining small molecule tyrosine kinase inhibitors with bevacizumab is being explored (Table 1).[43] Of particular importance are combinations with trastuzumab in HER2-positive breast cancer as erbB2 amplification has been shown to increase VEGF secretion by breast cancer cells.[44]

Toxicity

Because bevacizumab is a humanized monoclonal antibody, allergic reactions necessitating discontinuation of the drug are relatively infrequent.[45] The most common toxicities of bevacizumab are hypertension and proteinuria.[20,46] The hypertension is due to a decrease in endothelial nitric oxide synthase activity, resulting in lower nitric oxide levels which lead to vasoconstriction and elevated blood pressure. Treatment for hypertension is instituted before starting bevacizumab.[47] Once the patient is receiving bevacizumab, blood pressure should be monitored closely and appropriate antihypertensive therapy instituted if hypertension occurs or worsens. Interestingly, development of hypertension during treatment with bevacizumab may serve as prognostic factor for clinical outcome in patients receiving bevacizumab.[47] However, patients who develop hypertension are also at risk for proteinuria, which can develop into the nephrotic range, necessitating discontinuation of the bevacizumab.

Other common side effects include bleeding, problems with wound healing, bowel perforation and serious arterial thromboembolic events, including transient ischemic attack, cerebrovascular accident, angina, and myocardial infarction. Most of the bevacizumab-associated bleeding is epistaxis which is easily controlled.[20] However, serious hemoptysis has been reported in patients receiving bevacizumab for metastatic non-small-cell lung carcinoma.[15] Bevacizumab should not be used in patients receiving full dose anticoagulation or with a history of a bleeding diathesis.[45]

Bevacizumab has been associated with delayed wound healing, as well as dehiscence, ecchymosis, surgical site bleeding, and wound infection.[48] Elective surgery should be postponed until at least 40 days after the cessation of bevacizumab. Postoperative re-initiation of bevacizumab should not take place until the surgical incision has fully

Table 1 Bevacizumab combinations with targeted agents currently under investigation

PI	Type of study	Regimen	Second target	Clinical trials.gov ID	Patient population
Genentech	Phase II	sunitinib + paclitaxel + Bevacizumab terminated due to toxicity)	VEGFR	NCT00434356	Metastatic breast cancer
Miller[38]	Phase II	bevacizumab + sorafenib (terminated after enrollment of 18 pts due to toxicities)	VEGFR	NCT00632541	Metastatic breast cancer
AMGEN	Randomized phase II	paclitaxel + bevacizumab ± AMG706	Multiple kinases, including VEGFR	NCT00356681	HER2-negative metastatic breast cancer
Yardly	Randomized phase II	paclitaxel + bevacizumab ± everolimus	mTOR kinase	NCT00915603	HER2-negative metastatic breast cancer
Dickler[43]	Phase II	erlotinib + bevacizumab (this combination had little efficacy[43])	EGFR RTK	NCT00054132	Metastatic breast cancer
Erickson V	Non-comparative phase II	docetaxel + bevacizumab ± trastuzumab	HER2 RTK	NCT00364611	Her2-positive metastatic breast cancer
Glaxo	Phase II	lapatinib + bevacizumab	HER2 RTK	NCT00444535	Metastatic HER2 positive breast cancer
Genentech	Phase Ib	PI3Kinase inhibitor GDC-0941 in combination with paclitaxel and bevacizumab	PI3 kinase	NCT00960960	Metastatic breast cancer
Dickler[43]	Phase III	tamoxifen or letrozole + bevacizumab	Estrogen receptor	NCT00601900	Stage III or IV breast cancer

Notes: The studies that combine the anti-VEGF antibody bevacizumab with VEGFR-TKIs have been terminated due to toxicity. In addition, the combination of the EGFR-inhibitor erlotinib with bevacizumab has been reported and this combination had only limited therapeutic activity.

healed and at least 28 days after surgery to prevent increased wound healing complications.

Gastrointestinal perforation has been reported in a small percentage of patients treated with bevacizumab for colorectal cancer.[14,49,50] Perforation typically presented with abdominal pain with constipation and vomiting. Gastrointestinal perforations are rare in patients with breast cancer. Even when breast cancer patients have peritoneal implants or a high volume of visceral disease perforations occur in less than 3%.[51] Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation.

Serious arterial and venous thromboembolic events have been reported in patients receiving bevacizumab. Patients at highest risk of arterial thromboembolic events are those with a prior history of an arterial thrombotic event and patients over the age of 65. Bevacizumab should be permanently discontinued in patients who develop thrombotic events during treatment.

There has been concern that bevacizumab would cause hemorrhagic complications in patients with brain metastases. However, a recent analysis of several hundred patients with brain metastases who had received bevacizumab showed that the administration of bevacizumab did not significantly affect the risk of intracranial hemorrhage for patients with brain metastases from epithelial cancers.[52] In fact, the combination of avastin with carboplatinum is currently under investigation as a potentially active regimen in women with brain metastases from breast cancer (NCT01004172).

Less common complications include osteonecrosis of the jaw and reversible posterior leukoencephalopathy syndrome (RPLS). RPLS may be associated with hypertension and symptoms include headache, seizure, lethargy, confusion, blindness, and other visual and neurologic disturbances.[53,54] It usually resolves with discontinuation of bevacizumab and control of any associated hypertension. Osteonecrosis of the jaw is significantly increased in patients receiving bevacizumab in conjunction with bisphosphonates.[55,56]

Toxicities can be more frequent when bevacizumab is used in combination with chemotherapy or in the neo-adjuvant setting.[57] In a pre-operative study of cisplatin and bevacizumab, 37% of the patients with triple-negative breast cancer achieved a Miller-Payne 4 or 5 remission.

However 11% of the patients could not complete the treatment due to toxicities, including hypertension, pulmonary embolus and tinnitus or hearing loss. Additionally, this treatment combination led to significant rates of surgical complications, including wound breakdown, implant loss, hematomas and persistent seromas.[58]

Conclusions

A common theme in all combinations that included bevacizumab in breast cancer is that addition of the anti-VEGF antibody improves overall response rates and delays time to progression, but does not improve overall survival and does not seem to alter the course of the disease as dramatically as trastuzumab does in HER2-positive breast cancer. This points to the need to identify those patients who may benefit the most from this potentially toxic and costly treatment. Validated biomarkers for selecting cancer patients that should be considered for antiangiogenic therapy are needed.[59] Potential biomarkers such as VEGF polymorphisms or treatment responses such as hypertension, circulating angiogenic molecules or collagen IV all have some promise of helping to predict benefit and/or toxicity.

In the search for valid biomarkers, urine VEGF levels, tumor VEGF and thrombospondin 2 expression[38] as well as mutations in p53 and Ras were not predictors of response to bevacizumab.[60,61] However, in a retrospective analysis of ECOG2100, the presence of a germline single nucleotide polymorphism in the 5-prime untranslated region of VEGF-A, −2578AA and −1154AA showed a correlation with overall improved survival, as did the presence of greater than grade 2 hypertension.[62] Whether the differences in treatment outcomes are large enough to warrant stratification of patients according to these criteria, however, will need to be clarified in further studies.

Another unresolved, yet highly pressing issue is the emergence of resistance to antiangiogenic therapy, as almost all patients will eventually progress on bevacizumab, even if they achieved an initial response. In contrast to trastuzumab, which is continued even when chemotherapeutic agents are changed to address disease progression, there are no data to support the continued use of bevacizumab after disease progression. Indeed, a note of caution comes from recent laboratory observations about the prolonged use of angiogenesis inhibitors at a point of disease progression, and the use of PFS as an endpoint for the evaluation of angiogenesis inhibitors. While tumor-bearing mice treated with angiogenesis inhibitors experienced tumor shrinkage, their survival was reduced, and tumor invasiveness and metastasis were actually enhanced, leading to hypotheses that angiogenesis inhibition might facilitate the extravasation of tumor cells into a metastatic site or promote the creation of metastatic niches.[63,64]

The field of angiogenesis inhibitors for the treatment of breast cancer is young, and bevacizumab is currently under investigation internationally in over 100 clinical trials for all subtypes of stage II through IV breast cancer, mostly in combination with classical chemotherapy or newly developed targeted agents. Further validation of genetic biomarker profiles will likely aid in developing models to predict which patients will experience improved survival as a result of a bevacizumab-containing regimen.

Acknowledgments/disclosures

The authors report no conflicts of interest in this work.

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