Commentary

# Recent translational research: antiangiogenic therapy for breast cancer – where do we stand?

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## Abstract

The central importance of angiogenesis and our understanding of how new blood vessels are formed have led to the development of novel antiangiogenic therapies. Although the number of agents in development has grown exponentially, only one phase III trial in breast cancer has been completed. In that study the addition of bevacizumab to capecitabine did not extend the progression-free survival of patients with refractory disease as compared with capecitabine monotherapy. Early enthusiasm for antiangiogenic therapy must give way to clinical reality. Our challenge now is to exploit better the activity of antiangiogenic agents seen in the early clinical studies.

**Keywords:** angiogenesis, breast cancer, matrix metalloproteinase inhibitor, vascular endothelial growth factor

## Introduction

Normal vasculature is quiescent in healthy adults with each endothelial cell dividing once every 10 years; active angiogenesis is required only for wound healing, endometrial proliferation, postlactational mammary gland involution, and pregnancy. In contrast, tissue remodeling and angiogenesis are crucial for the growth and metastasis of breast cancer, providing an attractive therapeutic target [1]. The central importance of angiogenesis and our understanding of how new blood vessels are formed have led to novel therapies designed to interrupt this process (see http://www.angiogenesis.org or http://cancernet.nci.nih.gov for a detailed list of agents in development). Although the number of ongoing phase I and II trials has grown rapidly, few have been reported in the peer-reviewed literature. To date only one phase III trial in breast cancer has been completed.

Antiangiogenic agents may be conceptually categorized as follows: endothelial toxins, which specifically target endothelial antigens; growth factor/receptor antagonists, which thwart signaling of proangiogenic growth factors; protease inhibitors, which interfere with the action of proteases that are critical for invasion; and natural inhibitors, which stimulate or mimic endogenous inhibitors of angiogenesis. In addition, several chemotherapeutic agents routinely employed in breast cancer treatment have true antiangiogenic activity. Clinical experience with representative agents in each category is reviewed.

## Endothelial toxins

Disruption of endothelial cell chemotaxis and migration interferes with angiogenesis. The integrins, particularly $\alpha_v\beta_3$, provide critical attachment between the migrating endothelial cell and the extracellular matrix [2]; $\alpha_v\beta_3$ also localizes matrix metalloproteinase (MMP)-2 to the membrane of endothelial cells in the leading podosomes of new vessels, providing carefully targeted matrix destruction [3]. Vitaxin™ (Medimmune, Gaithersburg, MD, USA), a humanized monoclonal antibody recognizing $\alpha_v\beta_3$ (also known as the vitronectin receptor), inhibits endothelial proliferation *in vitro* and tumor growth *in vivo* [4]. In phase I trials Vitaxin™ was well tolerated but had limited activity [5,6]. Imaging tumor vasculature with $^{99m}$Tc Vitaxin™ was unsuccessful in one pilot study including at least one patient with $\alpha_v\beta_3$ positive melanoma [7]. Phase II trials are ongoing.



2ME2 = 2-methoxyestradiol; MMP = matrix metalloproteinase; VEGF = vascular endothelial growth factor.

## Growth factor antagonists

Angiogenesis requires stimulation of vascular endothelial cells through the release of angiogenic peptides, of which the vascular endothelial growth factor (VEGF) is the most potent. VEGF is a highly conserved, homodimeric, secreted, heparin-binding glycoprotein, the dominant isoform of which has a molecular weight of about 45 kDa [8]. The biologic effects of VEGF are mediated through binding to one of three endothelial surface receptors – VEGF-R1 (flt-1), VEGF-R2 (flk-1/kdr), and VEGF-R3; binding to the coreceptor neurophilin enhances signaling [9,10]. Although the VEGF receptors share considerable overlap in ligand binding, downstream effector interaction and biologic function, predominant actions have been identified. VEGF-R1 promotes differentiation and vascular maintenance [11]; VEGF-R2 induces endothelial cell mitogenesis and vascular permeability [12]; and VEGF-R3 stimulates lymphangiogenesis [13,14].

Bevacizumab (Avastin™; Genentech, South San Francisco, CA, USA), a humanized monoclonal antibody directed against VEGF-A, inhibits growth of human tumors in animal models [15]. A phase II study of bevacizumab monotherapy conducted in 75 patients with previously treated metastatic breast cancer [16] reported a 9.3% objective response rate with 17% of patients responding or stable at 22 weeks; four patients continued therapy without progression for over 12 months. Bevacizumab both alone and in combination with chemotherapy was well tolerated, with hypertension, proteinuria, thrombosis, and bleeding being the most commonly reported toxicities [17,18].

A recently reported phase III trial randomly assigned 462 patients with anthracycline- and taxane-refractory disease to receive capcitabine with or without bevacizumab; the primary end-point was progression-free survival as assessed by an independent review facility. As expected, bevacizumab therapy induced hypertension, proteinuria, and minor mucosal bleeding but these toxicities were rarely severe; 12% of patients in each group discontinued therapy because of toxicity. Combination therapy significantly increased the response rates whether designated by the independent review facility (9.1% versus 19.8%; $P = 0.001$) or the local investigators (19.1% versus 30.2%; $P = 0.006$). Because many of the excess responses in the combination group were relatively short-lived, progression free survival was similar in both groups (4.17 versus 4.86 months; hazard ratio = 0.98) [19]. Analysis of primary tumor samples for pathologic factors correlating with response to bevacizumab is ongoing. Initial results were limited by the small number of patients contributing samples but did not clearly identify a subset more likely to benefit [20]. A phase III trial (E2100) comparing paclitaxel, administered weekly for 3 out of 4 weeks, without or without bevacizumab in chemo-naïve patients with metastatic breast cancer is ongoing.

## Protease inhibitors

Degradation of the basement membrane and surrounding stroma by the MMPs is crucial for direct tissue invasion and angiogenesis. MMP inhibitors significantly curtail primary breast tumor growth and establishment of metastases in preclinical xenograft models but they fail to shrink large, well established tumors [21]. Nonetheless, previous MMP inhibitors were tested largely in patients with metastatic disease. Whether administered as monotherapy or in combination with cytotoxic agents, the results were nearly uniform. MMP inhibitors had little activity in advanced disease, leading to termination of clinical development of several agents [22–26]. The most successful clinical application of MMP inhibitors was hypothesized to be in patients with micrometastatic disease. However, two adjuvant pilot trials demonstrated intolerable musculoskeletal toxicity [27,28], making chronic administration of potentially therapeutic doses in the adjuvant setting implausible. The critical question, that regarding whether it is possible to separate inhibition of MMPs important in cancer progression from those whose inhibition produces joint toxicity, remains.

## Endogenous antiangiogenics

A naturally occurring metabolite of estradiol, namely 2-methoxyestradiol (2ME2), has a dual mechanism of action [29,30]: it acts as an antiproliferative drug, exerting its effect directly on the tumor cell compartment, and as an antiangiogenic drug, acting on tumor vasculature. Recent studies suggested that 2ME2 uses the extrinsic pathway for induction of apoptosis. 2ME2 upregulates death receptor 5 expression *in vitro* and *in vivo,* rendering cells more sensitive to the death receptor 5 ligand, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Inhibition of death receptor signaling by a dominant-negative Fas-associated death domain severely attenuates the 2ME2-induced apoptosis [31].

The first phase I trial of 2ME2 was conducted in patients with previously treated metastatic breast cancer [32]. 2ME2 was administered orally once (200–1000 mg/day; cohorts 1–5) or twice daily (200–800 mg every 12 hours; cohorts 6–9). Maximum tolerated dose was not reached. Metabolism was variable, with a half-life of approximately 10–12 hours. No objective responses were produced although prolonged disease stabilization was achieved in several patients. A second phase I study combined 2ME2 with docetaxel [33]. Treatment was well tolerated, with no detectable pharmacokinetic interaction. An overall response rate of 20% was reported; an additional 40% of patients had stable disease. In both phase I trials conversion to 2-methoxyestrone, an inactive metabolite, was significant, with 2-methoxyestrone concentrations generally 10-fold higher than 2ME2 levels. 2ME2 levels were substantially below those required for activity based on preclinical models. A new formulation with increased

bioavailability and activity in animal models is expected to re-enter clinical trials in 2004.

## Antiangiogenic chemotherapy

The intense interest in angiogenesis has also lead to a re-examination of the activity of many established cytotoxic agents. Several chemotherapeutic agents used routinely in breast cancer treatment have known antiangiogenic activity [34]. Maximal antiangiogenic activity typically requires prolonged exposure to low drug concentrations, exactly counter to maximum tolerated doses administered when optimal tumor cell kill is the goal. A number of recent reports confirm the importance of dose and schedule in preclinical models. In most models, the combination of low, frequent dose chemotherapy plus an agent that specifically targets the endothelial cell compartment controls tumor growth much more effectively than cytotoxic therapy alone [35–39]. These studies suggest that activated endothelial cells may be more sensitive, or even selectively sensitive, to protracted low-dose chemotherapy compared with other types of normal cells, thus creating a potential therapeutic window. Such selective sensitivity has been confirmed for several agents [40]. The antiangiogenic effect reported with low dose cyclophosphamide or microtubule agents may be due to induction of thrombospondin-1, a potent and endothelial-specific inhibitor of angiogenesis [41].

Thus far, few clinical trials have directly tested antiangiogenic schedules of chemotherapy. Nonetheless, the limited clinical evidence is intriguing. Remissions can be induced, albeit infrequently, in patients resistant to taxane therapy administered on an every 3-week basis by administering lower doses weekly [42]. E1199, a recently completed phase III adjuvant trial, compares paclitaxel with docetaxel weekly versus every 3 weeks and will determine the value of antiangiogenic taxane schedules.

The European Organization for Research and Treatment of Cancer studied two CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) regimens [43]: a classic 28-day regimen incorporating daily oral cyclophosphamide for 14 days and a modified intravenous schedule with bolus cyclophosphamide every 3 weeks. Overall response rate and survival clearly favored the classic regimen. Although generally viewed as a test of dose intensity (the classic regimen delivered higher total doses of both cyclophosphamide and 5-fluoruracil), that study may also be considered a test of an antiangiogenic versus bolus schedule. Superiority of the classic regimen in the adjuvant setting has also been suggested in a retrospective study of two groups of patients treated at different institutions [44]. A phase II study of low dose methotrexate (2.5 mg twice daily for 2 days each week) and cyclophosphamide (50 mg/day) in patients with previously treated metastatic breast cancer found an overall response rate of 19% (an additional 13% of patients were stable for 6 months or more). Serum VEGF levels decreased in all patients remaining on therapy for at least 2 months but this did not correlate with response [45]. Several ongoing trials in both the metastatic and adjuvant setting are investigating this regimen further.

## Conclusion

Our challenge now is to exploit better the activity of antiangiogenic agents seen in the early clinical studies. Thus far, antiangiogenic agents have been employed as general therapies given on a population basis, rather than as targeted therapies given to patients with a specific molecular phenotype. We must develop ways to select those patients who are most likely to benefit from each antiangiogenic agent. Perhaps even more importantly, the best time to intervene with an antiangiogenic agent may be earlier in the course of disease. Angiogenic pathways become more numerous and redundant as breast cancer progresses [46]. Given such redundancy, it is unlikely that inhibition of a single factor or pathway would produce a sustained clinical effect in patients with previously treated, highly refractory disease. As such, future trials must focus on patients with less advanced disease.

Early enthusiasm for antiangiogenic therapy, justified by impressive preclinical data, has given way to clinical reality – genes are not proteins are not cells are not tissues are not organs are not mice are not patients. Although the early rampant enthusiasm has been dampened, cautious optimism rightly remains. It now seems certain that antiangiogenic therapies will be integrated into routine clinical practice. To believe otherwise would be to assume that angiogenesis is both biologically crucial yet therapeutically unimportant, which is an unlikely paradox.

## Competing interests

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