VOLUME 23 · NUMBER 8 · MARCH 10 2005

JOURNAL OF CLINICAL ONCOLOGY

REVIEW ARTICLE

Angiogenesis of Breast Cancer

Bryan P. Schneider and Kathy D. Miller

J Clin Oncol 23:1782-1790. © 2005 by American Society of Clinical Oncology

From the Division of Hematology-Oncology, Indiana University, Indianapolis, IN.

Submitted December 22, 2004; accepted December 30, 2004.

Supported in part by the Breast Cancer Research Foundation (K.D.M.).

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Bryan P. Schneider, MD, Division of Hematology-Oncology, Indiana University, 535 Barnhill Dr, RT-473 Indianapolis, IN 46202; e-mail: bpschnei@iupui.edu.

0732-183X/05/2308-1782/$20.00

DOI: 10.1200/JCO.2005.12.017

PRECLINICAL EVIDENCE OF ANGIOGENESIS IN BREAST CANCER

Angiogenesis, the process of new blood vessel formation, plays a central role in both local tumor growth and distant metastasis in breast cancer.[1] Extensive laboratory data suggest that angiogenesis plays an essential role in breast cancer development, invasion, and metastasis. Hyperplastic murine breast papillomas[2] and histologically normal lobules adjacent to cancerous breast tissue[3] support angiogenesis in preclinical models, suggesting that angiogenesis precedes transformation of mammary hyperplasia to malignancy. Transfection of tumor cells with angiogenic stimulatory peptides has been shown to increase tumor growth, invasiveness, and metastasis. Conversely, transfection of tumor cells with inhibitors of angiogenesis decreases growth and metastasis.[4]

The matrix metalloproteinase (MMP) family of enzymes degrades the basement membrane and extracellular matrix and is associated with a family of endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Under normal physiologic conditions, the MMPs and TIMPs exist in an exquisite balance. This balance is disrupted during active angiogenesis. Expression of MMPs increases with the progression from benign to preinvasive, invasive, and metastatic breast cancer and is associated with increasing histologic tumor grade. Microscopic metastases are growth restricted and remain dormant until they undergo an angiogenic switch, presumably a result of further mutation. This angiogenic switch often results in increased expression of MMPs.[4]

Hypoxia is a key signal for the induction of angiogenesis. Hypoxia-inducible factors (HIF-1 and HIF-2) are heterodimeric transcription factors consisting of α and β subunits. The β subunit is constitutively expressed while the α subunit is protected from degradation only under hypoxic condition.[5,6] HIF-1α expression progressively increases from normal breast tissue to usual ductal hyperplasia to ductal carcinoma-in-situ to invasive ductal carcinoma. HIF-1α expression is higher in poorly differentiated than in well-differentiated lesions and is associated with increased proliferation and expression of the estrogen receptor and vascular endothelial growth factor (VEGF).[7] Similarly, expression of carbonic anhydrase IX, an HIF-1α-dependant enzyme important in pH regulation, is associated with worse relapse-free and overall survival in patients with invasive breast cancer.[8,9]

CLINICAL EVIDENCE OF ANGIOGENESIS IN BREAST CANCER

Clinicopathologic correlations also confirm the central role of angiogenesis in breast cancer progression. Fibrocystic lesions with the highest vascular density are associated with a greater risk of breast cancer.[10] Microvessel density (MVD) was shown to be highest with histopathologically aggressive ductal carcinoma-in-situ lesions[11] and associated with increased VEGF expression.[12] High MVD in premalignant lesions have been associated with high risk of future breast cancer.[10] Also, high MVD in invasive disease has been correlated with a greater likelihood of metastatic

disease[13] and a shorter relapse-free and overall survival in patients with node-negative breast cancer.[14]

Multiple angiogenic factors are commonly expressed by invasive human breast cancers; at least six different proangiogenic factors have been identified with the 121-amino acid isoform of VEGF predominating.[15] Carriers of the $C^{936}T$ allele in the VEGF gene were more frequent among controls (29.4%) than among a cohort of breast cancer patients (17.6%), implying a protective effect for carriers of the variant polymorphism.[16] A recent report also demonstrated that this same polymorphism is associated with an improved metastasis-free survival time in patients that have low-grade breast cancer.[17] VEGF expression has also been found to correlate with risk and outcomes in breast cancer. Several studies have found an inverse correlation between VEGF expression and overall survival in both node-positive and node-negative breast cancer.[18,19] Increased VEGF expression has also associated with impaired response to tamoxifen or chemotherapy in patients with advanced breast cancer.[20] Recently, VEGF expression has been successfully quantified via immunohistochemistry in breast cancer tumor specimens.[21] The expression and intensity of expression were found to correlate with a significantly inferior outcome of breast cancer.

AGENTS USED TO INHIBIT ANGIOGENESIS IN BREAST CANCER—CHALLENGES

The challenges of developing antiangiogenic agents and measuring their efficacy are not unique to the treatment of breast cancer. Familiarity with the distinct biologic nature of these agents is essential for the interpretation of clinical trials. Unlike cytotoxic agents that are measured by their ability to kill tumors directly, trials designed to evaluate these agents may require a paradigm shift. Because of the differences in mechanism of action, these agents must be delivered in a fashion to seek the biologically optimal rather than maximally tolerated dose. It may also be important to deliver chronic rather than intermittent therapy and strive to achieve induction of tumor dormancy rather than tumor cell kill as a therapeutic goal. Thus, measuring response rates in a traditional manner may not be appropriate and surrogate end points may be more predictive of therapeutic efficacy. Soluble measures of angiogenesis as well as emerging imaging technology may provide the solution to these challenges and are currently being studied.

AGENTS USED TO INHIBIT ANGIOGENESIS IN BREAST CANCER—THE CLASSICAL AGENTS

The use of antiangiogenic agents may not be a novel concept in the treatment of breast cancer.[22] Tamoxifen, initially thought to be merely a competitive inhibitor of estradiol, may also have estrogen-independent mechanisms of action.[23] Tamoxifen inhibits VEGF and fibroblastic growth factor–stimulated embryonic angiogenesis in the chick chorioallantoic membrane model. This effect was not reversed by excess estradiol, suggesting that the antiangiogenic mechanism is not dependent on estradiol concentration or estrogen receptor content.[24,25] Treatment with tamoxifen resulted in a more than 50% decrease in the endothelial density of viable tumor and an increase in the extent of necrosis in MCF-7 tumors growing in nude mice.[26] The inhibition of angiogenesis was detected before measurable effects on tumor volume.[27] In a study using differential display technology to assess gene expression in tumor and normal breast tissue from two patients, brief treatment with tamoxifen resulted in downregulation of CD36, a glycoprotein receptor for matrix proteins thrombospondin-1 and collagen types I and IV.[28]

Several chemotherapeutic agents used routinely in breast cancer treatment have known antiangiogenic activity.[29] 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.[30] Several reports confirmed the importance of dose and schedule in preclinical models. The combination of low, frequent dose chemotherapy plus an agent that specifically targets the endothelial cell compartment (TNP-470 and anti-VEGF-2) controlled tumor growth much more effectively than the cytotoxic agent alone.[31-33]

Thus far, few clinical trials have tested antiangiogenic schedules of chemotherapy, so-called metronomic therapy.[34] Metronomic dosing implements a frequent dosing schema at doses much lower than the maximum tolerated dosage. Preclinical data suggests that the mechanism responsible for the antiangiogenic effect is the induction of increased plasma levels of thrombospondin-1 (a potent and endothelial specific inhibitor of angiogenesis).[35] A phase II study of low-dose methotrexate (2.5 mg twice daily for 2 days each week) and cyclophosphamide (50 mg daily) 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 did not correlate with response.[36] 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 by in vitro work.[37] The Dana-Farber/Harvard Cancer Center (Boston, MA) is currently leading a phase II randomized study of metronomic low-dose cyclophosphamide and methotrexate with or without bevacizumab (Avastin; Genentech, South San Francisco, CA) in women with metastatic breast cancer. Twenty-six patients have

been enrolled thus far with no grade 3 or 4 toxicity to date (Harold J. Burstein, personal communication, December 2004).

AGENTS USED TO INHIBIT ANGIOGENESIS IN BREAST CANCER—THE CONTEMPORARY AGENTS

Agents Targeting the VEGF Ligand

Bevacizumab is the most mature therapeutic specifically designed to disrupt angiogenesis. Bevacizumab is a humanized monoclonal antibody directed against the VEGF-A ligand. It is US Food and Drug Administration–approved for the first-line treatment of metastatic colorectal cancer when given in combination with fluorouracil and irinotecan, based on the demonstration of an overall survival benefit over standard chemotherapy alone.[38] Bevacizumab has also demonstrated promising activity in renal cell carcinoma.[39,40]

In breast cancer, phase II studies in women who had previously progressed on at least one anthracycline- or taxane-based regimen revealed clinical activity.[41] Therapy was generally well tolerated; no significant bleeding episodes were noted, although it should be mentioned that patients were screened for intracranial metastases and those with brain metastases were excluded based on phase I experience with hemorrhage from unrecognized intracranial metastases. Phase II trials have also combined bevacizumab with a variety of other agents including vinorelbine[42] and docetaxel[43] in the refractory metastatic setting. A phase III trial testing bevacizumab has also recently been reported. This study randomly assigned 462 patients with anthracycline- and taxane-refractory, metastatic breast cancer to receive capecitabine with or without bevacizumab.[44] Hypertension requiring treatment (17.9% *v* 0.5%), proteinuria (22.3% *v* 7.4%), and thromboembolic events (7.4% *v* 5.6%) were more frequent in patients receiving bevacizumab. The primary end point of this study was time to progression and there was no evidence of improvement in the bevacizumab arm. The combination, however, significantly increased the response rates (9.1% *v* 19.8%; $P = .001$). Although attempts to correlate VEGF RNA overexpression (by in-situ hybridization) and response in this study were unsuccessful, the sample size was too small for a definitive conclusion.[45]

Bevacizumab has also been tested in the neoadjuvant setting. In one study, women were treated with docetaxel with or without bevacizumab. Eligible patients had locally unresectable breast cancer with or without distant metastasis. Patients whose disease responded underwent definitive surgery followed by four cycles of doxorubicin/cyclophosphamide and tamoxifen (if hormone receptor–positive). There were five complete clinical responses and 24 partial responses, and the therapy was generally well tolerated.[46] The National Cancer Institute also recently reported the preliminary results of a neoadjuvant pilot study evaluating bevacizumab (with several correlative end points) in women with inflammatory breast cancer. Patients received bevacizumab alone for the first cycle followed by six cycles of bevacizumab with doxorubicin and docetaxel. After completion of chemotherapy, eight of 13 patients experienced a confirmed partial response. There was also evidence of a decrease in vascular permeability on dynamic contrast-enhanced magnetic resonance imaging after the first cycle of bevacizumab monotherapy.[47]

There is preclinical and clinical rationale to support the combination of bevacizumab with trastuzumab. HER-2 appears to play a role in the regulation of VEGF.[48] An in-vitro study demonstrated increased HIF-1α and VEGF mRNA expression in HER-2-overexpressing cell lines.[49] In another preclinical study, exposure to trastuzumab significantly decreased VEGF in HER-2-overexpressing cells.[50] In-vivo experiments have shown reduction in xenograft volume using a combination of trastuzumab and bevacizumab compared with single-agent control.[50] In a cohort of 611 patients with primary breast cancer and a median follow-up of $>$ 50 months, there was a significant positive association between HER-2 and VEGF expression.[51] A recently reported phase I trial has evaluated the tolerability of the combination of bevacizumab with trastuzumab. In this trial, it was determined that the coadministration of these two humanized monoclonal antibodies did not alter the pharmacokinetics of either agent. Clinical responses were observed in five of nine patients, including one patient with prior disease progression on chemotherapy plus trastuzumab.[52]

There is also rationale to support simultaneous blockade of VEGF and the epidermal growth factor receptor (EGFR) pathways. The EGFR also appears to regulate VEGF[53,54] and several studies have demonstrated that blockade of the EGFR resulted in an antiangiogenic effect.[55,56] Furthermore, data have suggested that an increased production of VEGF represents one mechanism by which tumor cells escape anti-EGFR monoclonal antibody therapy.[57] One study has tested the strategy of combining bevacizumab and erlotinib (EGFR tyrosine kinase inhibitor) in metastatic breast cancer. This combination demonstrated activity and also found that changes in circulating endothelial cells and circulating tumor cells may correlate with response to this combination.[58]

A large, international phase III trial led by the Eastern Cooperative Oncology Group (E2100) compared paclitaxel with or without bevacizumab in chemotherapy-naïve patients with metastatic breast cancer. Women in this study received paclitaxel at 90 mg/m^2 weekly for 3 of 4 weeks with or without bevacizumab at 10 mg/kg every 2 weeks. This trial completed accrual in late May of 2004 and enrolled over 700 women. If positive, this trial will serve as a proof of concept for antiangiogenesis agents

in breast cancer. An ongoing trial led by the North Central Cancer Treatment Group is testing the combination of docetaxel, capecitabine, and bevacizumab as first-line chemotherapy in patients with metastatic breast cancer. There is also a planned Eastern Cooperative Oncology Group adjuvant feasibility trial evaluating bevacizumab in combination with dose-dense doxorubicin and cyclophosphamide followed by paclitaxel in women with node-positive breast cancer. Results of these studies are eagerly awaited.

Agents Targeting the VEGF Receptor

Receptor tyrosine kinase inhibitors. SU011248 (Pfizer, La Jolla, CA) is an inhibitor of receptor tyrosine kinases for VEGF receptor (VEGFR) -1, VEGFR-2, platelet-derived growth factor receptor (PDGFR), c-kit, and Flt-3. SU011248 has demonstrated preclinical activity in breast cancer models.[59,60] Furthermore, physiologic imaging in a breast cancer model during treatment with SU011248 revealed that [^{11}C] carbon monoxide and [^{18}F] fluoromethane imaging may be a useful biologic surrogate for this agent.[61] A phase II study of SU011248 in women with anthracycline and taxane-resistant metastatic breast cancer is currently ongoing. Preliminary toxicity data are available for 22 patients and the most frequently reported drug-related adverse events of any grade included diarrhea (32%), nausea (27%), fatigue (23%), and hypertension (14%). Preliminary efficacy data are available for 23 patients and there were four partial responses (two confirmed) and five patients with stable disease to date (K. Miller, unpublished data).

PTK787 (Novartis, Basel, Switzerland) is a pan-VEGF, PDGFR, c-kit, and c-Fms receptor tyrosine kinase inhibitor. It inhibited the growth of a broad panel of carcinomas in rodent models, with histologic examination revealing inhibition of microvessel formation.[62,63] Patients with a variety of advanced cancers have received this agent and it has been generally well tolerated. The Hoosier Oncology Group has recently activated a phase I/II study of PTK787 in combination with trastuzumab in patients with newly diagnosed HER-2-overexpressing, locally recurrent, or metastatic breast cancer. Two randomized, double-blind, phase III trials in patients with metastatic colorectal cancer are also ongoing.

ZD6474 (AstraZeneca, Boston, MA) is an inhibitor of VEGFR-2 and the EGFR tyrosine kinase. In a cohort of 7,12-dimethylbenz[a]anthracene-treated rats, there was inhibition of the formation of atypical ductal hyperplasia and carcinoma-in-situ by more than 95% and no invasive disease was seen when they received ZD6474.[64] A phase II trial of this agent in women with previously treated metastatic breast cancer was recently reported. It was generally well tolerated, with 26% of patients experiencing a rash (but none worse than grade 2). There were no objective responses and one patient had stable disease.[65] This was, however, a heavily pretreated population with a median of four prior chemotherapeutic agents.

Ribozymes. Angiozyme (Ribozyme Pharmaceuticals Inc, Boulder, CO) is a chemically stabilized ribozyme which targets the VEGFR-1 mRNA. A phase I/II study was undertaken in patients with refractory solid tumors and this agent was generally well tolerated.[66] A phase II trial in pretreated metastatic breast cancer patients has also been performed. Although there was evidence of biologic activity with a decrease in serum VEGFR-1 levels (in patients that had detectable baseline levels), there were no objective responses.[67]

MMP Inhibitors

Marimastat (British Biotech, Annapolis, MD) is an oral MMP inhibitor. A pilot feasibility study of this drug evaluated patients with high-risk, node-negative or node-positive breast cancer. Marimastat was given either as a single agent following completion of adjuvant chemotherapy or concurrently with tamoxifen. Arthralgia and arthritis were the most commonly reported toxicities. Six patients (19%) who received the 5-mg (twice daily) dose and 11 patients (35%) who received the 10-mg (twice daily) dose discontinued marimastat therapy due to toxicity. Trough plasma levels were rarely within the target range for biologic activity (40 to 200 ng/mL). These findings were discouraging, as the toxicity prohibited the maintenance of plasma levels with the target range.[68] E2196 was a phase III trial of 190 patients with metastatic breast cancer who had responding or stable disease after six to eight cycles of first-line chemotherapy for metastatic disease. Patients were randomly assigned to receive marimastat or a placebo after the completion of chemotherapy. There was no significant difference in median progression-free survival (4.7 *v* 3.1 months; $P = .16$) or overall survival (26.6 *v* 24.7 months; $P = .86$). Musculoskeletal toxicity was again an important toxicity. In that study, higher trough plasma marimastat levels, at month 1 or 3, were associated with a greater risk for progression and death.[69]

Natural Inhibitors of Angiogenesis

2-methoxyestradiol (2-ME; Entremed, Rockville, MD), a natural derivative of estradiol with limited affinity for the estrogen receptor, has both direct antitumor and antiangiogenic activity. The first phase I trial of 2-ME evaluated 31 patients with previously treated metastatic breast cancer. Seventeen patients had stable disease after the first treatment period and the therapy was generally well tolerated.[70] A phase I study of 2-ME plus docetaxel in patients with metastatic breast cancer has also been performed. The overall response rate was 20% (including one complete response) and an additional 40% of patients had stable disease. Concurrent therapy with docetaxel and 2-ME was well tolerated and did not alter the pharmacokinetics of

docetaxel or 2-ME.[71] In these studies, however, 2-ME serum levels were well below those required for activity based on preclinical models. A new formulation with improved bioavailability has now entered clinical trials.

Summary

Certainly the agents discussed here do not represent standard therapies for breast cancer patients currently. If we hope to optimize the possibility that these agents will eventually make an impact in the treatment of breast cancer, then we must foresee the potential obstacles that await us. Some of these hurdles and possible strategies to overcome them are discussed in the following sections.

RESISTANCE TO INHIBITORS OF ANGIOGENESIS

Because normal endothelial cells are genetically stable, antiangiogenic therapy was initially theorized to be a treatment "resistant to resistance."[72] Initial xenograft studies supported these predictions—widespread activity, limited toxicity, no resistance.[73] For a time it was argued that disease control, if not outright cure, was close at hand. Regrettably, the notion that antiangiogenic therapy was "resistant to resistance" proved not to be the case. Possible explanations follow.

Endothelial Cell Heterogeneity

One reason for the theoretical "resistance to resistance" theory was the belief that the endothelium was genetically stable in contradistinction to the surrounding tumor, which was known to have frequent mutations.[74] Mounting evidence now supports the concept that there is significant heterogeneity to the endothelium of vessels involved in angiogenesis, as well as sporadic mutations that they share with the parent tumors. Developing endothelium is dynamic and capable of differential gene expression based on the physiologic requirements and microenvironment of the associated tissue. A recent study found that 15% to 85% of the endothelial cells of the vasculature in non-Hodgkin's lymphoma tumors carried chromosomal abnormalities that were also harbored in the lymphoma cells.[75]

Angiogenic Factor Heterogeneity

There is growing evidence for a role for DNA polymorphisms for both pro- and antiangiogenic factors. Interindividual variability in drug response may be related to variations in host genes important in drug metabolism or transport. In addition to conferring a better or worse response to therapy, these polymorphisms may also play an important role in the development of cancers and the permissibility of early metastasis.[76] This is plausible in a number of ways, such as less effective DNA repair mechanisms, more efficient ability to create tumor blood supplies, and a quicker or slower ability to metabolize anticancer drugs, among others. A variety of polymorphisms in genes known to be important in the angiogenesis pathway have been shown to correlate with likelihood of developing breast cancer[16] as well as to serve as an important prognostic marker.[17,77] Collectively, these data suggest that considerable angiogenic heterogeneity is hard-wired into individual patients, and that polymorphic genes may play an important functional role in angiogenesis and breast cancer.

Tumor Cell Heterogeneity

Invasive cancers commonly express multiple angiogenic factors, and from a clinical standpoint, this heterogeneity occurs at an early point in time. Multiple different proangiogenic factors have been identified in primary breast tumors.[15] Genetic instability may result in modulation of both the amount and type of proangiogenic factors expressed in a tumor.[78] Tumor heterogeneity may imply more than just heterogeneity of proangiogenic factors. It has been shown that disruption of p53 in tumor cells reduces sensitivity to antiangiogenic metronomic therapy.[79]

Impact of the Tumor Microenvironment

Preclinical tumor models have demonstrated that orthotopic tumors have higher VEGF expression as well as more robust growth when compared with subcutaneous tumors.[80] Culture systems have also demonstrated that the medium conditioned by malignant cells provides a more stable environment for tumor and vessel growth than those conditioned by nonmalignant cells.[81] As many pro- and antiangiogenic factors are contained in or released from the extracellular matrix, differential sensitivity based on site of disease may be anticipated. For example, treatment with the matrix metalloproteinase inhibitor batimastat had different effects on tumor progression and growth depending on the site of tumor implantation.[82] The tumor microenvironment may also affect the delivery of the drug.[83]

Compensatory Responses to Treatment

It seems reasonable to expect VEGF production to increase in response to treatment with the pure antiangiogenics as well. Indeed, VEGF levels increased after therapy with doxorubicin and a VEGFR tyrosine kinase inhibitor.[84] Hypoxia may be chronic due to consumption/diffusion limitations or periodic resulting from transient reductions in tumor blood flow (so-called cyclic hypoxia).[85] It is reasonable to expect that these compensatory responses will be invoked by human cancers undergoing antiangiogenic attack.

Angiogenesis-Independent Tumor Growth

Vessel cooption, growth by intussusception, vascular mimicry, and vasculogenesis may decrease a tumor's dependence on classical angiogenesis. Multiple studies from tumor models have demonstrated the ability of

tumors to rely on these alternative methods to obtain the necessary blood supply when classical angiogenesis is not permitted.[86-95] Recent data also suggests that inflammatory breast cancer relies almost entirely on vasculogenesis as opposed to angiogenesis, apparently due to the inability of the cancer cells to bind endothelial cells.[96] If classic angiogenesis is not the predominant mechanism by which a tumor gets its blood supply, can antiangiogenic therapy be expected to succeed? This question still requires answering at the clinical level, but preclinical models have begun to explore the relative resistance of alternative blood supplies to antiangiogenic agents. It has been demonstrated that cancers characterized by vasculogenic mimicry are resistant to the antiangiogenic agent endostatin.[97] In contrast, bone marrow–derived endothelial cells remain sensitive to VEGF-targeting therapy.[98]

Pharmacokinetic Resistance

Preclinical studies of novel agents often fail to anticipate the dynamic nature of interactions between drug, host, and tumor. It is a peculiar failing of modern science that the study of simple—indeed, simplistic—model systems at the molecular or cellular level is considered more scientific than the study of complex, whole systems. And yet the failure of many antiangiogenic agents clearly occurs at exactly the higher-order levels of complexity seen in whole systems. These failures represent what might be termed pharmacokinetic resistance: the inability to deliver the right dose of a biologically active agent to the right cells for the right period of time.

OVERCOMING RESISTANCE TO ANTIANGIOGENIC THERAPY

Understanding the potential mechanisms of antiangiogenic resistance suggests several possible means to ameliorate or bypass such resistance.

Combine Antiangiogenic Agents With Standard Chemotherapy Regimens

Extensive preclinical data support a combined approach, with multiple antiangiogenic and chemotherapeutic agents having additive or synergistic combinatorial activity.[31,32,99,100] The mechanistic rationale for many of these combinations is poorly understood, and not intuitive, as both radiotherapy and chemotherapy depend on an effective blood supply for therapeutic efficacy. A potential explanation may lie in the inherent inefficiency of the tumor vasculature. Antiangiogenic therapy normalizes flow initially resulting in improved tissue oxygenation and decreased interstitial pressure, increasing delivery of cytotoxic agents.[101]

Combine Multiple Antiangiogenic Agents

As tumor progression is associated with expression of increasing numbers of proangiogenic factors, the use of multiple antiangiogenic agents to simultaneously attack this multiply redundant process may thwart resistance to individual agents. This approach is, of course, not unique to antiangiogenic therapy, having previously been used to limit resistance to cytotoxic, antimicrobial, and antiviral therapies. The combination of antiangiogenic agents has been tested in preclinical models with success.[102,103]

Combine Antiangiogenic Agents With Other Biologically Targeted Agents

Combined blockade of the VEGF and EGFR[58] pathways as well as the VEGF and HER-2 pathways[52] are actively being studied. As our understanding of tumor biology improves, concomitant blockade of other pathways will certainly be explored as well.

Use Antiangiogenic Therapy As Adjuvant Therapy

It is rare that a treatment is more effective for large rather than for small tumors. Tumor progression results in resistance to all anticancer therapies. One means of thwarting the development of drug resistance is to treat cancers when they are small. The adjuvant setting is the logical place to accomplish this goal. The use of antiangiogenics as adjuvant therapy has its own potential barriers. The toxicity of chronic antiangiogenic therapy remains largely unexplored, as is the toxicity of combinations of chemotherapy with antiangiogenic therapy. Although intuitively, the impact of angiogenesis inhibition is expected to be greatest in patients with micrometastatic disease, proof of this concept will require commitment of substantial human and financial resources to a randomized adjuvant trial.

Use Antiangiogenic Therapy As Targeted Therapy

Antiangiogenic therapy has been applied as a general therapy given on a population basis, rather than as a targeted therapy given to patients with a specific molecular phenotype. It is reasonable to ask whether we can call failure to respond to a therapy resistance if the target at which the therapy is aimed is not present in the tumor. If a patient's tumor does not express VEGF and therefore fails to respond to an anti-VEGF therapy, is the tumor resistant or is the therapy merely misguided? As insensitivity due to lack of therapeutic target results in resistance at the patient level, proper targeting is a means of overcoming such resistance. Ideal targets are biologically relevant, reproducibly measurable, and definably correlated with clinical benefit.

CONCLUSION

In breast cancer there is not yet definitive evidence for the efficacy of agents that specifically target angiogenesis. The

reason for this lack of success is likely two-fold. First, we have yet to optimize the strategies to overcome the mechanisms of resistance described above. Secondly, the trials designed to date have not allowed us to observe the true possibilities that these agents might provide since most trials have taken place in patients with refractory, progressive, metastatic disease. One route to optimize the efficacy of these targeted agents is to better understand their biology. As such, it is imperative to appropriately identify the target if we hope to hit it. In addition, we must become more astute at recognizing the correlations between the biology and clinical outcomes. This will require a concerted effort to perform tissue collection for testing as part of the development of these antiangiogenic agents. In addition, the design of new generation trials (such as E2100) which are testing the efficacy of these therapies in a less refractory setting, as well as trials which are targeting multiple pathways, will also hopefully shed light on the true potentials of these agents. It is by these avenues that we might most rapidly uncover the future possibilities of these agents.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

REFERENCES

1. Folkman J: Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182-1186, 1971

2. Brem SS, Gullino PM, Medina D: Angiogenesis: A marker for neoplastic transformation of mammary papillary hyperplasia. Science 195:880-882, 1977

3. Jensen HM, Chen I, DeVault MR, et al: Angiogenesis induced by ''normal'' human breast tissue: A probable marker for precancer. Science 218:293-295, 1982

4. Miller K, Sledge GW: Dimming the blood tide: Angiogenesis, antiangiogenic therapy and breast cancer, in Nabholtz JM (ed): Breast Cancer Management Application of Clinical and Translational Evidence to Patient Care (ed 2nd). Philadelphia, PA, Lippincott Williams & Wilkins, 2003, pp 287-308

5. Salceda S, Caro J: Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 272:22642-22647, 1997

6. Wang GL, Jiang BH, Rue EA, et al: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 92:5510-5514, 1995

7. Bos R, Zhong H, Hanrahan CF, et al: Levels of hypoxia-inducible factor-1 alpha during breast carcinogenesis. J Natl Cancer Inst 93:309-314, 2001

8. Chia SK, Wykoff CC, Watson PH, et al: Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. J Clin Oncol 19: 3660-3668, 2001

9. Wykoff CC, Beasley NJ, Watson PH, et al: Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res 60:7075-7083, 2000

10. Guinebretiere JM, Le Monique G, Gavoille A, et al: Angiogenesis and risk of breast cancer in women with fibrocystic disease. J Natl Cancer Inst 86:635-636, 1994

11. Guidi AJ, Fischer L, Harris JR, et al: Microvessel density and distribution in ductal carcinoma in situ of the breast. J Natl Cancer Inst 86:614-619, 1994

12. Guidi AJ, Schnitt SJ, Fischer L, et al: Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. Cancer 80:1945-1953, 1997

13. Weidner N, Semple JP, Welch WR, et al: Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. N Engl J Med 324:1-8, 1991

14. Weidner N, Folkman J, Pozza F, et al: Tumor angiogenesis: A new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 84:1875-1887, 1992

15. Relf M, LeJeune S, Scott PA, et al: Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 57:963-969, 1997

16. Krippl P, Langsenlehner U, Renner W, et al: A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer 106:468-471, 2003

17. Langsenlehner U, Samonigg H, Krippl P: Vascular endothelial growth factor gene polymorphism decreases the risk for breast cancer metastasis. Breast Cancer Res Treat 88:S72, 2004 (abstr 1090)

18. Gasparini G, Toi M, Gion M, et al: Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 89:139-147, 1997

19. Gasparini G, Toi M, Miceli R, et al: Clinical relevance of vascular endothelial growth factor and thymidine phosphorylase in patients with node-positive breast cancer treated with either adjuvant chemotherapy or hormone therapy. Cancer J Sci Am 5:101-111, 1999

20. Foekens JA, Peters HA, Grebenchtchikov N, et al: High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. Cancer Res 61:5407-5414, 2001

21. Ragaz J, Miller K, Badve S, et al: Adverse association of expressed vascular endothelial growth factor (VEGF), Her2, Cox2, uPA and EMSY with long-term outcome of stage I-III breast cancer (BrCa). Results from the British Columbia Tissue Microarray Project. Proc Am Soc Clin Oncol 23:8, 2004 (abstr 524)

22. Miller KD, Sweeney CJ, Sledge GW, Jr.: Redefining the target: Chemotherapeutics as antiangiogenics. J Clin Oncol 19:1195-1206, 2001

23. Wiseman H: Tamoxifen: New membrane-mediated mechanisms of action and therapeutic advances. Trends Pharmacol Sci 15:83-89, 1994

24. Gagliardi A, Collins DC: Inhibition of angiogenesis by antiestrogens. Cancer Res 53:533-535, 1993

25. Gagliardi AR, Hennig B, Collins DC: Antiestrogens inhibit endothelial cell growth stimulated by angiogenic growth factors. Anticancer Res 16:1101-1106, 1996

26. Haran EF, Maretzek AF, Goldberg I, et al: Tamoxifen enhances cell death in implanted MCF7 breast cancer by inhibiting endothelium growth. Cancer Res 54:5511-5514, 1994

27. Lindner DJ, Borden EC: Effects of tamoxifen and interferon-beta or the combination on tumor-induced angiogenesis. Int J Cancer 71: 456-461, 1997

28. Silva ID, Salicioni AM, Russo IH, et al: Tamoxifen down-regulates CD36 messenger RNA levels in normal and neoplastic human breast tissues. Cancer Res 57:378-381, 1997

29. Sweeney C, Sledge G: Chemotherapy agents as antiangiogenic therapy. Cancer Conference Highlights 3:2-4, 1999

30. Slaton JW, Perrotte P, Inoue K, et al: Interferon-alpha-mediated down-regulation of angiogenesis-related genes and therapy of bladder cancer are dependent on optimization of biological dose and schedule. Clin Cancer Res 5:2726-2734, 1999

31. Browder T, Butterfield CE, Kraling BM, et al: Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 60:1878-1886, 2000

32. Klement G, Baruchel S, Rak J, et al: Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 105:R15-R24, 2000

33. Wild R, Dings R, Subramanian I, et al: Carboplatin selectively induces the VEGF stress response in endothelial cells: Potentiation of antitumor activity by combination treatment with antibody to VEGF. Int J Cancer 110:343-351, 2004

34. Hanahan D, Bergers G, Bergsland E: Less is more, regularly: Metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J Clin Invest 105:1045-1047, 2000

35. Bocci G, Francia G, Man S, et al: Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. Proc Natl Acad Sci U S A 100:12917-12922, 2003

36. Colleoni M, Rocca A, Sandri MT, et al: Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: Antitumor activity and correlation with vascular endothelial growth factor levels. Ann Oncol 13:73-80, 2002

37. Bocci G, Nicolaou KC, Kerbel RS: Protracted low-dose effects on human endothelial cell proliferation and survival in vitro reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res 62:6938-6943, 2002

38. Hurwitz H, Fehrenbacher L, Novotny W, et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335-2342, 2004

39. Hainsworth J, Sosman J, Spigel D, et al: Phase II trial of bevacizumab and erlotinib in patients with metastatic renal carcinoma (RCC). Proc Am Soc Clin Oncol 23:381, 2004 (abstr 4502)

40. Yang JC, Haworth L, Sherry RM, et al: A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 349:427-434, 2003

41. Sledge G, Miller K, Novotny W, et al: A phase II trial of single-agent rhumab VEGF (recombinant humanized monoclonal antibody to vascular endothelial cell growth factor) in patients with relapsed metastatic breast cancer. Proc Am Soc Clin Oncol 19:3a, 2000 (abstr 5c)

42. Burstein H, Parker L, Savoie J, et al: Phase II trial of the anti-VEGF antibody bevacizumab in combination with vinorelbine for refractory advanced breast cancer. Breast Cancer Res Treat 76:S115, 2002 (abstr 446)

43. Ramaswamy B, Rhoades C, Kendra K, et al: CTEP-sponsored phase II trial of bevacizumab (Avastin) in combination with docetaxel (Taxotere) in metastatic breast cancer. Breast Cancer Res Treat 82:S50, 2003 (abstr 224)

44. Miller K, Rugo H, Cobleigh M, et al: Phase III trial of capecitabine (Xeloda) plus bevacizumab (Avastin) versus capecitabine alone in women with metastatic breast cancer (MBC) previously treated with an anthracycline and a taxane. Breast Cancer Res Treat 76:S37, 2002 (abstr 36)

45. Hillan K, Koeppen H, Tobin P, et al: The role of VEGF expression in response to bevacizumab plus capecitabine in metastatic breast cancer (MBC). Proc Am Soc Clin Oncol 22:191, 2003 (abstr 766)

46. Overmoyer B, Silverman P, Leeming R, et al: Phase II trial of neoadjuvant docetaxel with or without bevacizumab in patients with locally advanced breast cancer. Breast Cancer Res Treat 88:S106, 2004 (abstr 2088)

47. Wedam S, Low J, Yang X, et al: A pilot study to evaluate response and angiogenesis after treatment with bevacizumab in patients with inflammatory breast cancer. Proc Am Soc Clin Oncol 23:21, 2004 (abstr 578)

48. Koukourakis MI, Giatromanolaki A, O'Byrne KJ, et al: bcl-2 and c-erbB-2 proteins are involved in the regulation of VEGF and of thymidine phosphorylase angiogenic activity in non-small-cell lung cancer. Clin Exp Metastasis 17:545-554, 1999

49. Laughner E, Taghavi P, Chiles K, et al: HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: Novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 21:3995-4004, 2001

50. Epstein M, Ayala R, Tchekmedyian N, et al: HER2-overexpressing human breast cancer xenografts exhibit increased angiogenic potential mediated by vascular endothelial growth factor (VEGF). Breast Cancer Res Treat 76:S143, 2002 (abstr 570)

51. Konecny GE, Meng YG, Untch M, et al: Association between HER-2/neu and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. Clin Cancer Res 10:1706-1716, 2004

52. Pegram M, Yeon C, Ku N, et al: Phase I combined biological therapy of breast cancer using two humanized monoclonal antibodies directed against HER2 proto-oncogene and vascular endothelial growth factor (VEGF). Breast Cancer Res Treat 88:S124, 2004 (abstr 3039)

53. Maity A, Pore N, Lee J, et al: Epidermal growth factor receptor transcriptionally up-regulates vascular endothelial growth factor expression in human glioblastoma cells via a pathway involving phosphatidylinositol 3′-kinase and distinct from that induced by hypoxia. Cancer Res 60:5879-5886, 2000

54. Clarke K, Smith K, Gullick WJ, et al: Mutant epidermal growth factor receptor enhances induction of vascular endothelial growth factor by hypoxia and insulin-like growth factor-1 via a PI3 kinase dependent pathway. Br J Cancer 84:1322-1329, 2001

55. Bruns CJ, Solorzano CC, Harbison MT, et al: Blockade of the epidermal growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to apoptosis of endothelial cells and therapy of human pancreatic carcinoma. Cancer Res 60:2926-2935, 2000

56. Petit AM, Rak J, Hung MC, et al: Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: Angiogenic implications for signal transduction therapy of solid tumors. Am J Pathol 151:1523-1530, 1997

57. Viloria-Petit A, Crombet T, Jothy S, et al: Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: A role for altered tumor angiogenesis. Cancer Res 61:5090-5101, 2001

58. Rugo H, Dickler M, Scott J, et al: Circulating endothelial cell (CEC) and tumor cell (CTC) analysis in patients (pts) receiving bevacizumab and erlotinib for metastatic breast cancer (MBC). Breast Cancer Res Treat 88:S142, 2004 (abstr 3088)

59. Abrams TJ, Murray LJ, Pesenti E, et al: Preclinical evaluation of the tyrosine kinase inhibitor SU11248 as a single agent and in combination with ''standard of care'' therapeutic agents for the treatment of breast cancer. Mol Cancer Ther 2:1011-1021, 2003

60. Murray LJ, Abrams TJ, Long KR, et al: SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. Clin Exp Metastasis 20:757-766, 2003

61. Miller K, Miller M, Mehrotra S, et al: The search for surrogates—physiologic imaging in a breast cancer xenograft model during treatment with SU11248. Breast Cancer Res Treat 82:S18, 2003 (abstr 38)

62. Drevs J, Hofmann I, Hugenschmidt H, et al: Effects of PTK787/ZK 222584, a specific inhibitor of vascular endothelial growth factor receptor tyrosine kinases, on primary tumor, metastasis, vessel density, and blood flow in a murine renal cell carcinoma model. Cancer Res 60:4819-4824, 2000

63. Wood JM, Bold G, Buchdunger E, et al: PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. Cancer Res 60:2178-2189, 2000

64. Heffelfinger SC, Yan M, Gear RB, et al: Inhibition of VEGFR2 prevents DMBA-induced mammary tumor formation. Lab Invest 84:989-998, 2004

65. Miller K, Trigo J, Stone A, et al: A phase II trial of ZD6474, a vascular endothelial growth factor receptor-2 (VEGFR-2) and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, in patients with previously treated metastatic breast cancer (MBC). Breast Cancer Res Treat 88:S240, 2004 (abstr 6060)

66. Weng D, Weiss P, Kellackey C, et al: Angiozyme pharmacokinetic and safety results: A phase I/II study in patients with refractory solid tumors. Proc Am Soc Clin Oncol 20:99a, 2001 (abstr 393)

67. Hortobagyi G, Weng D, Elias A, et al: ANGIOZYME treatment of stage IV metastatic breast cancer patients: Assessment of serum markers of angiogenesis. Breast Cancer Res Treat 76:S97, 2002 (abstr 362)

68. Miller KD, Gradishar W, Schuchter L, et al: A randomized phase II pilot trial of adjuvant marimastat in patients with early-stage breast cancer. Ann Oncol 13:1220-1224, 2002

69. Sparano JA, Bernardo P, Stephenson P, et al: Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy: Eastern Cooperative Oncology Group trial E2196. J Clin Oncol 22:4631-4638, 2004

70. Sledge G, Miller K, Haney L, et al: A phase I study of 2-methoxyestradiol (2ME2) in patients (pts) with refractory metastatic breast cancer (MBC). Proc Am Soc Clin Oncol 21:111a, 2002 (abstr 441)

71. Miller K, Murry D, Curry E, et al: A phase I study of 2-methoxyestradiol (2ME2) plus docetaxel (D) in patients (pts) with metastatic breast cancer (MBC). Proc Am Soc Clin Oncol 21:111a, 2002 (abstr 442)

72. Kerbel RS: A cancer therapy resistant to resistance. Nature 390:335-336, 1997

73. Boehm T, Folkman J, Browder T, et al: Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 390:404-407, 1997

74. Fidler IJ, Ellis LM: Neoplastic angiogenesis–not all blood vessels are created equal. N Engl J Med 351:215-216, 2004

75. Streubel B, Chott A, Huber D, et al: Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas. N Engl J Med 351:250-259, 2004

76. Evans WE, McLeod HL: Pharmacogenomics–drug disposition, drug targets, and side effects. N Engl J Med 348:538-549, 2003

77. Ghilardi G, Biondi ML, Cecchini F, et al: Vascular invasion in human breast cancer is correlated to T→786C polymorphism of NOS3 gene. Nitric Oxide 9:118-122, 2003

78. Cahill DP, Kinzler KW, Vogelstein B, et al: Genetic instability and darwinian selection in tumours. Trends Cell Biol 9:M57-M60, 1999

79. Kerbel RS, Yu J, Tran J, et al: Possible mechanisms of acquired resistance to anti-angiogenic drugs: Implications for the use of combination therapy approaches. Cancer Metastasis Rev 20:79-86, 2001

80. Gohongi T, Fukumura D, Boucher Y, et al: Tumor-host interactions in the gallbladder suppress distal angiogenesis and tumor growth: Involvement of transforming growth factor beta1. Nat Med 5:1203-1208, 1999

81. Liu W, Davis DW, Ramirez K, et al: Endothelial cell apoptosis is inhibited by a soluble factor secreted by human colon cancer cells. Int J Cancer 92:26-30, 2001

82. Low JA, Johnson MD, Bone EA, et al: The matrix metalloproteinase inhibitor batimastat (BB-94) retards human breast cancer solid tumor growth but not ascites formation in nude mice. Clin Cancer Res 2:1207-1214, 1996

83. Pluen A, Boucher Y, Ramanujan S, et al: Role of tumor-host interactions in interstitial diffusion of macromolecules: Cranial vs. subcutaneous tumors. Proc Natl Acad Sci U S A 98:4628-4633, 2001

84. Overmoyer B, Robertson K, Persons M: A phase I pharmacokinetic and pharmacodynamic study of SU5416 and adriamycin in inflammatory breast cancer. Breast Cancer Res Treat 69:284, 2001 (abstr 432)

85. Durand RE, LePard NE: Contribution of transient blood flow to tumour hypoxia in mice. Acta Oncol 34:317-323, 1995

86. Djonov V, Andres AC, Ziemiecki A: Vascular remodelling during the normal and malignant life cycle of the mammary gland. Microsc Res Tech 52:182-189, 2001

87. Folberg R, Hendrix MJ, Maniotis AJ: Vasculogenic mimicry and tumor angiogenesis. Am J Pathol 156:361-381, 2000

88. Kunkel P, Ulbricht U, Bohlen P, et al: Inhibition of glioma angiogenesis and growth in vivo by systemic treatment with a monoclonal antibody against vascular endothelial growth factor receptor-2. Cancer Res 61:6624-6628, 2001

89. Passalidou E, Trivella M, Singh N, et al: Vascular phenotype in angiogenic and non-angiogenic lung non-small cell carcinomas. Br J Cancer 86:244-249, 2002

90. Patan S, Munn LL, Jain RK: Intussusceptive microvascular growth in a human colon adenocarcinoma xenograft: A novel mechanism of tumor angiogenesis. Microvasc Res 51:260-272, 1996

91. Patan S, Tanda S, Roberge S, et al: Vascular morphogenesis and remodeling in a human tumor xenograft: Blood vessel formation and growth after ovariectomy and tumor implantation. Circ Res 89:732-739, 2001

92. Stessels F, Van den Eynden G, Van der Auwera I, et al: Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. Br J Cancer 90:1429-1436, 2004

93. Asahara T, Masuda H, Takahashi T, et al: Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 85:221-228, 1999

94. Bolontrade MF, Zhou RR, Kleinerman ES: Vasculogenesis plays a role in the growth of Ewing's sarcoma in vivo. Clin Cancer Res 8:3622-3627, 2002

95. Hattori K, Dias S, Heissig B, et al: Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med 193:1005-1014, 2001

96. Alpaugh M, Barsky S: The molecular basis of inflammatory breast cancer. Breast Cancer Res Treat 69:312, 2001 (abstr 563)

97. van der Schaft D, Seftor E, Hess A, et al: The differential effects of angiogenesis inhibitors on vascular network formation by endothelial cells versus aggressive melanoma tumor cells. Proc Am Assoc Cancer Res 44:696, 2003 (abstr 3046)

98. Lyden D, Hattori K, Dias S, et al: Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 7:1194-1201, 2001

99. Sweeney CJ, Miller KD, Sissons SE, et al: The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. Cancer Res 61:3369-3372, 2001

100. Teicher BA, Sotomayor EA, Huang ZD: Antiangiogenic agents potentiate cytotoxic cancer therapies against primary and metastatic disease. Cancer Res 52:6702-6704, 1992

101. Jain RK: Normalizing tumor vasculature with anti-angiogenic therapy: A new paradigm for combination therapy. Nat Med 7:987-989, 2001

102. Brem H, Gresser I, Grosfeld J, et al: The combination of antiangiogenic agents to inhibit primary tumor growth and metastasis. J Pediatr Surg 28:1253-1257, 1993

103. Scappaticci FA, Smith R, Pathak A, et al: Combination angiostatin and endostatin gene transfer induces synergistic antiangiogenic activity in vitro and antitumor efficacy in leukemia and solid tumors in mice. Mol Ther 3:186-196, 2001