Antiangiogenic Strategies and Agents in Clinical Trials

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ABSTRACT
The understanding that the growth of tumors depends on the acquisition of a blood supply has led to the development of new therapies for cancer and other angiogenic diseases based on inhibition of neovascularization. This review examines the role of angiogenesis in cancer progression and describes various strategies for interfering with this process. The developmental status of angiogenesis inhibitors in human clinical trials is presented, including their proposed mechanisms of action. Standard chemotherapeutic agents and angiogenesis inhibitors are compared, noting that different end points might need to be considered in clinical trials and that drug resistance may be less of a problem with antiangiogenic therapy than with conventional chemotherapy regimens. The suggestion is made that cytotoxic chemotherapy and angiogenesis inhibitors used in combination may produce complementary therapeutic benefits in the treatment of cancer. The Oncologist 2000;5(suppl 1):20-27

INTRODUCTION
Angiogenesis is a process through which new blood vessels develop from preexisting vessels. This dynamic process is tightly controlled by a large number of proangiogenic and antiangiogenic factors. Normal vasculature is quiescent, outside of certain physiological processes such as wound repair and the female menstrual cycle. The turnover time of these cells can be several years in duration [1]. In contrast, endothelial cells during neovascularization can proliferate with a turnover time of several days [2]. The understanding that the growth of tumors depends on angiogenesis has led to the development of novel strategies for treatment directed at tumor vasculature. Antiangiogenic compounds have had striking success in preclinical models, and new agents are rapidly entering into clinical trials. This article describes the current understanding of the principal pathways for angiogenesis and summarizes some of the types of agents available and their current clinical status. It will also address some of the difficulties in interpreting trials with drugs whose sole mechanism of action is thought to be the inhibition of angiogenesis. Finally, the possible future role these agents may play in the treatment of neoplastic disease will be discussed.

THE ROLE OF ANGIOGENESIS IN CANCER PROGRESSION
Current research suggests that each tumor arises from a single cell that has been transformed by one or more events. Such events include the activation of oncogenes and the absence or inactivation of specific tumor-suppressor genes. These transformed cells can form small clones, initially co-opting normal host vessels, growing to only several millimeters in size before their supply of nutrients becomes limited [3-6]. At this point, the tumor may lie dormant for prolonged periods (from months to years) until ultimately undergoing destruction by the immune system or switching to an angiogenic phenotype [2]. This “switch” involves a shift in the local equilibrium between negative and positive endogenous regulators of angiogenesis [2, 7]. The tumor cells may achieve this shift in several ways, including the overexpression of angiogenic factors, the recruitment of host cells (such as macrophages) that can produce their own angiogenic factors, the mobilization of angiogenic proteins from the extracellular matrix (ECM), or a combination of these processes [2]. If the production of proangiogenic factors is sufficiently robust, neighboring endothelial cells will be activated, leading to the sprouting of new capillaries.

The creation of new blood vessels occurs by a series of steps [2, 8]. An endothelial cell forming the wall of an existing small blood vessel is activated, makes matrix metalloproteinase (MMP) enzymes that break down the ECM, invades the matrix, and then begins to proliferate. Eventually, strings of new endothelial cells organize into hollow tubes, creating new networks of blood vessels that supply a given tissue with nutrients to promote growth.
and repair. The amount of new blood vessel growth can correlate with poor prognosis in several tumor types [9, 10]. Since the shedding of large numbers of tumor cells from the primary tumor may not begin until after the tumor has a sufficient network of blood vessels, angiogenesis may also correlate with metastatic potential. Destruction of the ECM through MMP activation is probably necessary to initiate the metastatic process. Microvessel density has been correlated with cancer invasion and metastasis in a number of human tumors including breast [11, 12], prostate [13], lung [10], esophageal [14], colorectal [15], endometrial [9], and cervical [16]. In addition, bone marrow angiogenesis was found to be of prognostic value in patients with multiple myeloma [17] and other hematopoietic malignancies [18].

Various proteins activate endothelial cell growth and movement including vascular endothelial growth factor (VEGF) [19], acidic and basic fibroblast growth factors (aFGF and bFGF), transforming growth factors-alpha and beta, angiogenin [20], epidermal growth factor [21, 22], scatter factor/hepatocyte growth factor [23, 24], placental growth factor [25], interleukin 8 [26-28], and tumor necrosis factor alpha [22, 29]. Some of the known naturally occurring inhibitors of angiogenesis include angiostatin [30], endostatin [31], interferon alfa [32], platelet factor 4 [33, 34], thrombospondin [35], transforming growth factor beta [2], 16 kD fragment of pro- lactin [36], and tissue inhibitor of metalloproteinase (TIMP), including TIMP-1, TIMP-2, and TIMP-3 [37, 38].

**Strategies for Blocking Angiogenesis**

Of the antiangiogenic drugs now in clinical trials, some were designed to directly target specific molecules involved in new blood vessel formation (e.g., SU5416, which inhibits VEGF-receptor signaling) while others indirectly inhibit endothelial cell function or response (e.g., inhibitors of MMP breakdown). The precise mechanism of action of some of these compounds is not known, although their antiangiogenic properties have been demonstrated in animal models or in vitro testing. Some drugs (e.g., thalidomide, as discussed below) appear to have both antiangiogenic properties and other mechanisms of action, in which case defining specific mechanisms of action in a given clinical situation may prove challenging. One strategy for interfering with the process of angiogenesis involves using agents that inhibit the proliferation or response of normal endothelial cells. Targets for this strategy include endoglin, integrins, and VEGF-receptor complexes. Another strategy involves blocking the growth factor mediated cell signaling pathways that stimulate angiogenesis using small molecule inhibitors of VEGF-receptor signaling pathways, antibodies to VEGF, dominant negative receptors that block the activity of VEGF, and agents that prevent the release or activation of FGF. The direct administration of endogenous angiogenic inhibitors such as angiostatin, endostatin, and the gene transfer of DNA that encodes for angiogenesis inhibitors, including angiostatin and platelet factor 4, are also being evaluated. Additional strategies for blocking angiogenesis involve administration of synthetic angiogenic inhibitors that specifically prevent endothelial cell division, such as synthetic derivatives of fumagillin or the inhibition of the endothelial cell mediated breakdown of the surrounding matrix. Targets for the latter strategy include enzymes that dissolve ECM and may initiate and promote angiogenesis, including interstitial collagenase (MMP-1), 72 kD gelatinase A/type IV collagenase (MMP-2), and 92 kD gelatinase B/type IV collagenase (MMP-9) [39]. Counterbalancing the effects of these enzymes are TIMP-1, TIMP-2, TIMP-3, and possibly TIMP-4, proteins that inhibit neovascularization [39, 40]. Although TIMPs have been shown to inhibit tumor metastasis in some in vivo models [41, 42], they are not directly suitable for pharmacological applications owing to their short half-life in vivo and large molecular weight. Nevertheless, pharmacological modulation of MMPs is currently being explored in the clinic.

Although the above strategies depict the antiangiogenic activities of compounds as distinct, their mechanisms of action often overlap. For example, the angiogenesis inhibitor angiostatin may be produced from plasminogen by MMP action [43, 44], and laminin-5 is specifically degraded by MMP-2 to produce a soluble chemotactic fragment [44, 45]. This suggests that angiostatin may be producing effects through direct inhibition of the endothelial cell as well as by interaction with mechanisms of tissue breakdown. In another case, thalidomide has been shown to inhibit angiogenesis, possibly through its modulation of integrins [46, 47], but it may act by directly binding DNA [48], stimulating T cell responses [49], and upregulating tumor antigens [50], among its other effects. Similarly, TNP-740, a fumagillin derivative in clinical trials, not only interferes with endothelial cell proliferation by inhibiting Ets-1 transcription factor expression [51] but also demonstrates immunosuppressive effects via inhibition of methionine aminopeptidase 2 [52] and acts as an inhibitor of membrane translocation of bioproteins [53].

Many commonly used oncological agents can modulate angiogenesis as a secondary mechanism of action. For example, the camptothecin analogs, 9-amino-20(S)-camptothecin, topotecan, and CPT-11, are inhibitors of topoisomerase I and have also been shown to decrease tumor-associated angiogenesis [54]. Paclitaxel, a microtubule inhibitor that is an active agent in the treatment of many different cancers, was shown to possess antiangiogenic properties independent of its antiproliferative action in vivo models [55, 56]. Thus, in many cases, multiple activities ascribed to individual agents confound our ability...
to determine precisely the contribution of angiogenesis inhibition to an agent’s efficacy.

**ANGIOGENESIS INHIBITORS IN CLINICAL TRIALS**

**Agents that Inhibit Endothelial Cells**

Several endogenous angiogenesis inhibitors or agents that directly inhibit endothelial cell proliferation are undergoing phase I/II clinical evaluation, either alone or in combination with chemotherapy (Table 1). These compounds are of low molecular weight, except for endostatin, which is a 20 kD C-terminal fragment of collagen XVIII. Phase II clinical trials in several tumor types are under way with thalidomide, despite its withdrawal from sale in 1961 after being held responsible for the birth of hundreds of phocomelus children. A recent phase II study of thalidomide has demonstrated its antitumor activity in patients with refractory multiple myeloma [57]. Although these compounds all directly inhibit endothelial cells, their molecular targets on endothelial cells are diverse. In particular, combretastatin is unique since it induces apoptosis in proliferating endothelial cells, leading to blood flow shutdown to tumors rather than to surrounding normal tissues.

**Agents that Block Activators of Angiogenesis**

Targeting VEGF directly interferes with the angiogenic process and thus allows for therapy specificity, unlike many of the other mechanisms of the antiangiogenic drugs currently under study. Ongoing clinical trials of agents that block the VEGF pathway are shown in Table 2. VEGF, an endothelial cell-specific mitogen normally produced during embryogenesis and adult life, is a significant mediator of angiogenesis in a variety of normal and pathological processes including tumor development. Eppenberger et al. reported that tumor VEGF level was the most important prognostic parameter among several markers of tumor angiogenesis [58]. SU5416, a small molecule inhibitor of the VEGF receptor Flk-1/KDR, is currently under clinical evaluation. To date, 69 patients have been enrolled in the first phase I clinical trial with SU5416; the drug has been found to be well tolerated, with stable disease observed in patients with Kaposi’s sarcoma (KS), non-small cell lung, colorectal, renal cell, and basal cell cancers [59]. Although the mechanism of action is cytostatic in nature, partial responses have been observed with SU5416. Recently, SU5416 has been shown to induce endothelial and tumor cell apoptosis in a xenograft model of colon cancer liver metastasis [60]. The ability of SU5416 to induce apoptosis in cancer cells that lack adequate vasculature may have contributed to the partial responses observed.

Interferon alfa has been commercially available for almost 15 years and has demonstrated an ~30% response rate in patients with AIDS-related KS with good prognostic factors [61-63].

### Table 1. Endogenous inhibitors and drugs that directly inhibit endothelial cells

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
<th>Trial</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNP-740</td>
<td>Synthetic analog of</td>
<td>Phase II for adults with advanced cancer with solid tumors; phase I</td>
<td>TAP Pharmaceuticals Inc., Deerfield, IL</td>
</tr>
<tr>
<td></td>
<td>fumagillin; inhibition of Ets-1</td>
<td>for patients with lymphomas and acute leukemias</td>
<td>Commercially available, Celsgene Corporation, Warren, NJ</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Possibly through modulation of integrins; exact mechanism unknown</td>
<td>Phase II with chemotherapy against solid tumors; adjuvant study in recurrent or metastatic colorectal cancer; pilot study in non-small cell lung cancer; phase II in graft versus host disease; phase II studies in refractory myeloma, urogenital cancers, KS, recurrent head and neck, and unfavorable karyotype AML/MDS</td>
<td>Commercially available, Celsgene Corporation, Warren, NJ</td>
</tr>
<tr>
<td>Squalamine</td>
<td>Inhibition of sodium-hydrogen exchanger NHE3</td>
<td>Phase I, solid tumors; phase II, non-small cell lung cancer and others</td>
<td>Magainin Pharmaceuticals, Plymouth Meeting, PA</td>
</tr>
<tr>
<td>Combretastatin phosphate</td>
<td>Induction of apoptosis in proliferating endothelial cells; tubulin destabilization</td>
<td>Phase I, solid tumors; phase II to begin mid-2000</td>
<td>OxiGene, Boston, MA</td>
</tr>
<tr>
<td>Vitaxin</td>
<td>Humanized monoclonal antibody to αVβ3 integrin present on endothelial cell surface</td>
<td>Phase I in advanced tumors completed; pilot phase II in leiomyosarcoma</td>
<td>Ixysys, Inc., La Jolla, CA</td>
</tr>
<tr>
<td>EMD121974</td>
<td>Small molecule blocker of αVβ3 integrin present on endothelial cell surface</td>
<td>Phase I/III in KS, brain tumors, and solid tumors</td>
<td>Merck KgAa, Darmstadt, Germany</td>
</tr>
<tr>
<td>Endostatin</td>
<td>20 kD C-terminal proteolytic fragment of collagen XVIII that potently inhibits endothelial cell proliferation and angiogenesis</td>
<td>Phase I, surrogate end point trial of Human Recombinant Endostatin in Patients with Advanced Solid Tumors</td>
<td>EntreMed, Rockville, MD</td>
</tr>
</tbody>
</table>

Abbreviations: AML = acute myeloid leukemia; KS = Kaposi’s sarcoma; MDS = myelodysplastic syndrome.
Agents that Block Extracellular Matrix Breakdown

Ongoing clinical trials of seven MMP inhibitors are shown in Table 3. Marimastat has been well tolerated in phase I studies in healthy volunteers who received short courses of treatment [71]. The symptoms of severe joint pain and muscle pain reported by many patients were reversible after discontinuation of the drug. A pooled analysis of six studies in colorectal, ovarian, and prostate cancer has demonstrated a dose-dependent biological effect. A total of 58% of patients responded at doses >50 mg twice a day. Potential clinical activity in pancreatic and gastric cancer has also been demonstrated in small phase II studies. These studies have demonstrated that cytotoxic chemotherapy can be safely combined with marimastat, and hence ongoing phase III studies are further investigating the utility of combination therapy with marimastat and cytotoxic therapy in the treatment of small cell lung cancer and pancreatic and gastric carcinoma [71]. A 15-patient, phase I pharmacokinetic study of AG3340 in combination with mitoxantrone and prednisone in patients with advanced prostate cancer has reported that the combination is well tolerated [72]. A phase I study has also reported the combination of AG3340, paclitaxel, and carboplatin as safe and well tolerated in the treatment of patients with advanced solid tumors [73]. Several phase III trials with this agent are ongoing, and results will be reported shortly. Bay 12-9566 has undergone phase I testing as a single agent [74] and is currently undergoing further testing in combination with standard doses of paclitaxel and carboplatin [75]. MM270 has completed single-agent phase I studies, and its administration with concomitant folinic acid is currently being explored [76].

Additional updated information on antiangiogenesis agents in clinical trials can be obtained through individual sponsors as well as on the National Cancer Institute Cancer Trial website, http://cancertrials.nci.nih.gov

**Table 2. Drugs that block activators of angiogenesis**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
<th>Trial</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU5416</td>
<td>Small molecule blocker of VEGF-receptor signaling through inhibition of KDR</td>
<td>Phase II/I in KS, advanced malignancies, and metastatic colorectal cancer; phase II in von Hippel-Lindau disease; phase III in advanced untreated colorectal and non-small cell lung cancers</td>
<td>Sugen, South San Francisco, CA</td>
</tr>
<tr>
<td>SU668</td>
<td>Small molecule blocker of VEGF, FGF, and PDGF receptor signaling</td>
<td>Phase I in selected advanced tumors</td>
<td>Sugen, South San Francisco, CA</td>
</tr>
<tr>
<td>PTK787/ZK22584</td>
<td>Inhibition of the VEGF receptor-kinase insert domain-containing receptor</td>
<td>Phase I in KS</td>
<td>Novartis Pharmaceuticals Corporation, East Hanover, NJ</td>
</tr>
<tr>
<td>Interferon alfa</td>
<td>Inhibition of bFGF and VEGF production among others</td>
<td>Phase I in mesothelioma; phase I/I in AML and lymphoproliferative diseases; phase II in renal cell, pancreatic, bladder, CML, gastric, neuroblastoma, CLL multiple myeloma, meningioma, recurrent H+N, colorectal; phase III melanoma, renal cell, melanoma, CML, lymphoma, and uterine*</td>
<td>Commercially available</td>
</tr>
<tr>
<td>Anti-VEGF antibody</td>
<td>Humanized mAb to VEGF</td>
<td>Phase II in metastatic renal cell cancer, advanced prostate cancer, non-small cell lung cancer, colorectal cancer, and other solid tumors</td>
<td>Genentech, Inc., South San Francisco, CA</td>
</tr>
</tbody>
</table>

*In combination with chemotherapy.
† In combination with retinoic acid, BCG = bacillus Calmette-Guérin, interleukin 2, or other biological agents.
Abbreviations: AML = acute myeloid leukemia; bFGF = basic fibroblast growth factor; CLL = chronic lymphocytic leukemia; CML = chronic myeloid leukemia; H+N = head and neck; KS = Kaposi’s sarcoma; mAb = monoclonal antibody; PDGF = platelet derived growth factor; VEGF = vascular endothelial growth factor.

treatment of hemangiomas [64], chronic myeloid leukemias [65], myelomas [66], melanomas [67], lymphomas [68, 69], and renal cell carcinomas [70].

**Angiogenesis Inhibitors Versus Standard Chemotherapy**

Unlike standard chemotherapy that targets tumor cells and other proliferating cells, angiogenesis inhibitors target dividing endothelial cells. Thus, antiangiogenic agents are not as likely to cause toxicities such as gastrointestinal symptoms and myelosuppression that are characteristic of standard chemotherapeutic regimens. In addition, drug resistance can be a major problem with chemotherapy.
agents since most cancer cells are genetically unstable and are therefore prone to mutations. In contrast, cancer cells are less likely to develop resistance to antiangiogenic agents since these agents target normal endothelial cells, which are more genetically stable. As demonstrated by preclinical and early clinical data, drug resistance has not been a major concern with angiogenesis inhibitors. Further, since normal vasculature in the adult is quiescent, the appropriate use of selective inhibitors of angiogenesis may be expected to confer a degree of specificity that is not obtainable with the nonspecific modalities of chemotherapy and radiation therapy and to allow for relatively nontoxic, long-term treatment of tumors.

Because antiangiogenic agents are expected to be cytostatic rather than cytotoxic, the proper end points of early clinical trials may have to differ from those of standard chemotherapy agents. For antiangiogenesis therapy, improved survival or increased time-to-disease progression (see the article “Clinical Strategy for the Development of Angiogenesis Inhibitors” in this volume) may be a more appropriate end point than evaluating objective response alone. In addition, issues related to treating children and women of childbearing potential—groups with ongoing physiological angiogenesis—would have to be considered.

Lastly, the use of antiangiogenesis therapy and cytotoxic chemotherapy are not mutually exclusive. Since the two therapeutic strategies are directed at different cellular targets, antiangiogenesis therapy may prove useful in combination with standard chemotherapy directed at tumor cells. As noted in this review, such trials are currently under way.

### Table 3. Drugs that block matrix breakdown

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
<th>Trial</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marimastat</td>
<td>Synthetic inhibitor that blocks TNF-α convertase; inhibitor of MMPs</td>
<td>Phase II in pancreas; phase III in non-small cell lung, small cell lung, and breast cancers; phase I/II in glioblastoma multiforme</td>
<td>British Biotech, Annapolis, MD</td>
</tr>
<tr>
<td>AG3340</td>
<td>Synthetic MMP inhibitor</td>
<td>Phase III non-small cell lung, hormone refractory prostate, pancreatic, gastric, and small cell lung cancers</td>
<td>Agouron Pharmaceuticals, Inc., La Jolla, CA</td>
</tr>
<tr>
<td>COL-3</td>
<td>Synthetic MMP inhibitor; tetracycline derivative</td>
<td>Phase I in solid tumors</td>
<td>Collagenex, Newtown, PA</td>
</tr>
<tr>
<td>Neovastat (AE941)</td>
<td>Naturally occurring MMP inhibitor</td>
<td>Phase III in non-small cell lung cancer</td>
<td>Aeterna, Sainte-Foy, Quebec, Canada</td>
</tr>
<tr>
<td>Bay 12-9566</td>
<td>Synthetic inhibitor of MMP-2 and -9</td>
<td>Phase III in pancreatic, ovarian cancers</td>
<td>Bayer, New Haven, CT</td>
</tr>
<tr>
<td>MMI270 (CGS27023A)</td>
<td>Synthetic MMP inhibitor</td>
<td>Phase I, colorectal cancer</td>
<td>Novartis, East Hanover, NJ</td>
</tr>
<tr>
<td>BMS-275291</td>
<td>Synthetic MMP inhibitor</td>
<td>Phase I</td>
<td>Bristol-Myers Squibb, Wallingford, CT</td>
</tr>
</tbody>
</table>

Abbreviations: MMP = matrix metalloproteinase; TNF-α = tumor necrosis factor alpha.

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