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## **Tumor Vasculature: The Achilles' Heel of Cancer ?**

Simone Mocellin

Department of Oncological and Surgical Sciences, Surgery Branch,  
School of Medicine, University of Padova, via Giustiniani 2, 35128 Padova, Italy

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**Abstract:** Given its pivotal role in growth and survival, the tumor vasculature represents an attractive target for anticancer therapy. Over the last few decades, rapid progress has been achieved in the understanding of tumor angiogenesis including signaling pathways and their regulation. This has enabled the development of numerous potentially effective vasculature-targeted anticancer drugs (VTAD), which are being tested in the clinical setting. In this review I will focus on the most promising and advanced drugs targeting the tumor vasculature, briefly summarizing their mechanism of action and the clinical results so far obtained.

**Key words:** cancer, tumor vasculature, angiogenesis, therapy, drugs, clinical trials

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### **Introduction**

Tumors require a functioning vascular network to provide tumor cells with oxygen and other nutrients and also to remove toxic waste products associated with cellular metabolism. This vasculature can be acquired, in part, by the incorporation of existing host blood vessels. Nonetheless, for continued growth and development, tumors must generate their own networks of microvessels through the process of neovascularization. In fact, it is widely accepted that no solid tumor can grow larger than a critical size of 1 mm<sup>3</sup> without developing a blood supply network. The neovasculature that is formed in tumors differs greatly from what is found in most normal adult tissue. It is primitive in nature, morphologically and functionally abnormal and typically unable to keep pace with the rapidly growing tumor cell mass. Thus, the vascular network inevitably fails to meet the nutritional needs of the tumor cells and this failure results in nutrient deprivation, oxygen deficiency and highly acidic conditions in certain regions within the tumor. Given its pivotal role in growth and survival, the tumor vasculature represents an attractive target for anticancer therapy. Two key approaches to targeting the tumor blood vessel network have been developed (Tortora *et al.*, 2004). The first approach aims to inhibit the tumor-initiated angiogenic process itself (antiangiogenic drugs). Strategies that have been tested include the use of agents that interfere with the delivery or export of angiogenic stimuli; antibodies that inhibit or inactivate angiogenic factors after their release; and inhibitors of receptor activity, tumor invasion, or endothelial cell proliferation. Many of these agents currently are undergoing clinical evaluation. The alternative approach involves the use of therapeutic agents to preferentially destroy the established tumor vessel network. These vasculature-disrupting drugs (VDD) differ from antiangiogenic agents not only in their mode of action but also in their therapeutic application. Whereas VDD are used in intermittent doses, antiangiogenic treatment is administered continually over months or years. At present, clinical trials involving lead VDD also are being conducted.

## **Tumor Vascularization and Angiogenesis**

Like in healthy tissues, tumor neovascularization may include angiogenesis, vasculogenesis and intussusception. Angiogenesis represents the process of new blood vessels sprouting from existing vessels. Recently, vasculogenesis, recruitment of circulating endothelial cells differentiated from stem cells into the newly formed blood vessels, has been found to be critical for tumor neovascularization (Lyden *et al.*, 2001). In addition, intussusception, a process of splitting large “mother” blood vessels into smaller “daughter” vessels, has been reported to participate in tumor vessel growth. Despite their ability to stimulate new blood vessel growth from the host, there are fundamental differences between tumor vessels and host tissue vessels. Morphologically, tumor vessels are irregular, heterogeneous and leaky. These features are considered as hallmarks of destruction of normal blood vessel integrity. The endothelial cells are disorganized and irregularly shaped, sometimes overlapped each other and luminal projections, which lead to abluminal sprouts. It has been reported that blood vessels in tumors consist of mosaic cell types including tumor cells (Folberg *et al.*, 2000). Although mural cells have been found on tumor vessels, they have unusually loose associations with endothelial cells. In addition, tumor vessels contain an abnormal basement membrane including changes in matrix protein composition, assembly and structures. Unlike normal blood vessels in a healthy tissue, there is no clear distinction between arterioles and venules among tumor vessels. As a result of this abnormal vessel architecture, blood flow in tumor vessels is chaotic. For example, a single vessel transports blood to distal tumor cells and removes it from the tumor tissue. Thus, the tumor tissue is relatively hypoxic with poorly oxygenated blood. The unusual leaky features of tumor vessels result in increased interstitial pressure that would further restrict fresh blood flow into the tumor tissue. Thus, normalization of tumor vessels and blood supply would improve drug delivery into the tumor tissue. It is also possible that normalization of leaky tumor vessels could prevent cancer metastasis by limiting the access of tumor cells into the circulation.

### *Signaling Pathways in Cancer Angiogenesis*

A finely tuned equilibrium exists between physiological anti-angiogenic and pro-angiogenic factors (Carmeliet, 2003). Angiogenesis is mostly mediated by vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) under hypoxic conditions due to tumor progression. Low pH, hypoglycemia and inflammation induced by tumor proliferation also promote angiogenesis. The angiogenic tumor phenotype is characterized by high microvessel density, elevated VEGF levels and is correlated to poor prognosis. During angiogenesis, endothelial cells are stimulated by various growth factors by binding to membrane receptors such as tyrosine kinase receptors (TKR). TKR that are directly involved in angiogenesis include receptors for VEGF, FGF, PDGF, Ang-1, angiopoietin-2 (Ang-2), hepatocyte growth factor (HGF), Eph and receptors belonging to the epithelial growth factor family. VEGF is the most potent inducer of angiogenesis and there are four VEGF receptors (VEGFR). Like the other TKR involved in angiogenesis, these receptors are expressed by endothelial cells and not by the tumor cells secreting the growth factors. TKR are not only activated by their own ligands but also by hormones, neurotransmitters and lymphokines.

### *VEGF and VEGF Receptors*

Angiogenesis is a fundamental developmental and adult physiological process, requiring the coordinated action of a variety of growth factors and cell-adhesion molecules in endothelial and mural cells. So far, VEGF-A and its receptors are the best-characterized signaling pathway in developmental

angiogenesis (Ferrara *et al.*, 2003). Loss of a single VEGF-A allele results in embryonic lethality. This pathway also has an essential role in reproductive and bone angiogenesis. Much research has also established the role of VEGF-A in tumor angiogenesis. VEGF-A binds to two receptor tyrosine kinases (RTK), VEGFR-1 (Flt-1) and VEGFR-2 (KDR, Flk-1). Of the two, it is now generally agreed that VEGFR-2 is the major mediator of the mitogenic, angiogenic and permeability-enhancing effects of VEGF-A. The significance of VEGFR-1 in the regulation of angiogenesis is more complex. Under some circumstances, VEGFR-1 may function as a “decoy” receptor that sequesters VEGF and prevents its interaction with VEGFR-2. However, there is growing evidence that VEGFR-1 has significant roles in haematopoiesis and in the recruitment of monocytes and other bone-marrow-derived cells that may home in on the tumor vasculature and promote angiogenesis. In addition, VEGFR-1 is involved in the induction of matrix metalloproteinases (MMP) and in the paracrine release of growth factors from endothelial cells. Thus the VEGFR-1-selective ligands VEGF-B and placental-like growth factor (PLGF) may also have a role in these processes. Furthermore, in some cases VEGFR-1 is expressed by tumor cells and may mediate a chemotactic signal, thus potentially extending the role of this receptor in cancer growth. VEGF-A gene expression is upregulated by hypoxia: in particular, the transcription factor hypoxia inducible factor (HIF), which operates in concert with the product of the von Hippel-Lindau (VHL) tumor suppressor gene (under normo-oxic conditions, the VHL protein targets HIF for ubiquitination and degradation), has a major role in such regulation. *In situ* hybridization studies demonstrate that VEGF-A messenger RNA is expressed in many human tumors. Renal cell carcinomas have a particularly high level of VEGF-A expression, consistent with the notion that inactivating VHL mutations occur in about 50% of such tumors, thus providing a further explanation for the responsiveness of this tumor type to a VEGF-A blockade. However, VEGF-A upregulation in tumors is not only linked to hypoxia or VHL mutations. Indeed, a very broad and diverse spectrum of oncogenes is associated with VEGF-A upregulation, including mutant Ras, ErbB-2/Her2, activated EGFR and Bcr-Abl. In 1993, investigators reported that a murine anti-human VEGF-A monoclonal antibody inhibited the growth of several tumor cell lines in nude mice, whereas the antibody had no effect on tumor-cell proliferation *in vitro* (Kim *et al.*, 1993). Subsequent studies have shown that many additional tumor cell lines, regardless of the tumor’s origin, are inhibited *in vivo* by the same anti-VEGF monoclonal antibody or other strategies, as described in the second part of this essay.

#### *Platelet-derived Growth Factor (PDGF) and Angiopoietins*

Other signaling molecules that have an established role in the development and differentiation of the vessel wall such as PDGF-B/PDGFR- $\beta$  and the angiopoietins (Ang), the ligands of the Tie2 receptor, may also be therapeutic targets (Yancopoulos *et al.*, 2000). PDGF-B is required for recruitment of pericytes and maturation of the microvasculature. Inhibition of PDGFR-signalling has been reported to result in a tumor microvascular tree that is particularly dependent on VEGF-mediated survival signals. Withdrawal of VEGF-A leads to endothelial apoptosis and vascular regression. In this context, newly formed vessels, whether they are tumor-associated or not, are particularly vulnerable to VEGF-A blockade, whereas mature vessels, covered by extracellular matrix and pericytes may be resistant to VEGF inhibitors and other antiangiogenic agents. Furthermore, recent studies have emphasized the significance of tumor-derived PDGF-A (and potentially PDGF-C) and PDGFR-signaling in the recruitment of an angiogenic stroma that produces VEGF-A and other angiogenic factors. Therefore, combining PDGF and VEGF inhibitors is an attractive anti-vascular and anti-tumor strategy. Ang-1 is required for further remodeling and maturation of the initially immature vasculature. Unlike mouse embryos lacking VEGF-A or VEGFR-2, embryos lacking Ang-1 or its receptor Tie2

develop a rather normal primary vasculature, but this vasculature fails to undergo effective remodeling. The generally accepted view is that Ang-1 is the major agonist for Tie2, whereas Ang-2 may act as an antagonist or a partial agonist. However, more recent evidence indicates that, unexpectedly, Ang-2 has a positive role, at least in tumor angiogenesis. Administration of Ang-2 inhibitors to tumor-bearing mice has been reported to result in delayed tumor growth, accompanied by reduced endothelial cell proliferation, consistent with an antiangiogenic mechanism. Therefore, inhibitors of Ang-2 may be candidates for clinical development.

#### *Axon-guidance Molecules*

Recently, the role of axon-guidance receptors and ligands in developmental angiogenesis has received much attention (Klagsbrun and Eichmann 2005). There are four main families: the neuropilins (NRP)/semaphorins, the ephrins, Robo/Slit and netrin/Unc5. Although the significance of these pathways in tumor angiogenesis is far from clear, there is emerging evidence that they have a role in some cancer models and therefore may be potential therapeutic targets. NRP1 and NRP2, previously shown to bind the collapsin/semaphorin family and implicated in axon guidance, are also receptors for the heparin-binding isoforms of VEGF-A and appear to potentiate the activation of VEGFR-2 by VEGF165. Therefore, NRP may participate in tumor angiogenesis as positive modulators of VEGF signaling in endothelial cells. Furthermore, NRP1 and NRP2 are expressed on the cell surface of several tumor cell lines that bind VEGF165 and display a chemotactic response to this ligand, suggesting a pro-tumor activity of NRP, with or without the involvement of VEGF RTK signaling. The ephrins and their tyrosine kinase Eph receptors are a large family, initially implicated in neuronal guidance during development and subsequently found to have activities in other cell types, including vascular cells. The earliest evidence for a role of this family in angiogenesis was that ephrin A1 mediates TNF-induced angiogenesis *in vivo*. Recent studies suggest a role for Eph/ephrin interactions in malignant tumor progression and angiogenesis. Soluble EphB4-expressing human melanoma A375 cells grown subcutaneously in nude mice showed reduced tumor growth compared with control tumors. Interfering with EphA signaling has been also reported to result in inhibition of angiogenesis in tumor models. Slits are secreted proteins that function as chemorepellents in axon guidance and neuronal migration through the Roundabout (Robo) receptor. Investigators reported that Slit2 is expressed in several tumor cell types and that Robo1 is localized to vascular endothelial cells. Moreover, recombinant Slit2 protein attracted endothelial cells and promoted tube formation. Finally, neutralization of Robo1 reduced microvessel density and growth of A375 cells transplanted in nude mice.

#### *Endogenous Negative Regulators of Angiogenesis*

Angiogenesis is a tightly regulated process and seems to be under the control of both positive and negative regulatory factors. Although several potential negative regulators of angiogenesis have been identified, relatively little is known about their role in the physiological regulation of angiogenesis (Sund *et al.*, 2005). Thrombospondin, a large multifunctional glycoprotein secreted by most epithelial cells in the extracellular matrix, inhibits angiogenesis associated with tumor growth and metastasis. Several fragments of larger proteins have been described as endogenous inhibitors of angiogenesis including endostatin, tumstatin and vasostatin. The most recently described endogenous inhibitor of angiogenesis is vasohibin, which seems to be derived from the endothelium and to operate as a feedback regulator. The precise mechanism of action of these proteins remains to be more clearly defined, although several hypotheses have been proposed, including that they bind to specific integrins in the case of endostatin and tumstatin.

#### *Bone-marrow-derived Cells and Angiogenesis*

An intensively debated issue in the oncology field is the role of bone-marrow-derived endothelial progenitor cells (EPC) to angiogenesis (Peters *et al.*, 2005). However, there is little doubt that bone-marrow-derived cells participate in angiogenic processes, at least as a source of angiogenic factors. After their isolation from human peripheral blood on the basis of cell-surface expression of CD34 and other endothelial markers, EPC were reported to differentiate *in vitro* into endothelial cells and appeared to be incorporated at sites of active angiogenesis in various animal models of ischaemia. These findings suggested that incorporation into the lumen of bone-marrow-derived endothelial precursor cells contributes to the growing vessels, complementing resident endothelial cells sprouting new vessels. Also, ischaemia and various cytokines, including VEGF and granulocyte-macrophage colony-stimulating factor (GM-CSF), were reported to mobilize EPC into sites of neovascularization. However, the precise contribution of these cells in various pathophysiological circumstances was not clearly defined. Subsequent studies have suggested that the contribution of such cells to angiogenesis is dependent on the experimental system employed. In the angiogenic-defective, tumor-resistant Id-mutant mice, EPC accounted for a large proportion of endothelial cells associated with xenografted tumors. Investigators proposed that mobilization of EPC from bone marrow requires angiogenic-factor-mediated activation of MMP-9, which leads to the release of the soluble KIT ligand. This ligand would in turn promote proliferation and motility of EPC within the bone-marrow microenvironment, thus creating permissive conditions for their mobilization into the peripheral circulation. However, spontaneous tumors occurring in Id-deficient mice in the tumor-prone PTEN +/- genetic background, the contribution of EPC was less significant. Furthermore, other investigators suggested that the percentage of EPC that are truly incorporated into a growing vessel wall is very low and that the majority of bone-marrow-derived cells homing in on the tumor vasculature are adherent perivascular mononuclear cells, which may contain angiogenic factors.

#### **Vasculature Targeted Anticancer Drugs**

Large numbers of vasculature targeted anticancer drugs (VTAD) with various mechanisms of action are currently under clinical development and in this review we will focus on the most promising and advanced drugs targeting the tumor vasculature briefly summarizing their mechanism of action and the clinical results so far obtained.

##### *Inhibition of the VEGF Pathway*

##### *Tyrosine Kinase Inhibitors*

##### *SU6668*

This compound has a wide spectrum of activity and inhibits the tyrosine kinase activity of PDGFR, FGFR1 and VEGFR2. It permits the regression of human tumor xenografts in mice with complete histological response following the rapid apoptosis of tumor microvessels. The maximum tolerated dose of SU6668 given orally, thrice daily under fed conditions, is 100 mg m<sup>-2</sup>. Because of the low plasma levels reached at this dose level, the efficacy of SU6668 as a single agent is not to be expected (Kuenen *et al.*, 2005).

##### *SU011248*

SU011248 inhibits VEGFR1, PDGFR and c-Kit, a receptor of the stem cell growth factor (SCF) implicated in malignant blood diseases. At higher concentrations, it inhibits another angiogenesis TKR, FGFR1. SU011248 was synergistic with radiotherapy in mouse models attaining tumor responses and

sustained tumor control. In a phase II study, 63 patients with renal cancer after failure of a cytokine received SU011248 that resulted in 21 (33%) partial responses and 23 (37%) stabilizations (Mancuso and Sternberg 2005); moreover, the 1-year survival was 65%. Interestingly, SU011248 has also achieved clinical activity in gastrointestinal stromal tumors (GIST). GIST are sensitive to STI-571 a specific c-Kit tyrosine-kinase inhibitor. After failure of STI-571, 98 patients (92 evaluated) received SU011248. Of those patients, 7 (8%) partial responses (PR) and 53 (58%) SD were observed. Efficacy of SU011248 is higher in patients without mutation in c-Kit or PDGFR-A, or a mutation in the exons 9, 13 and 14 of c-Kit or PDGFR-A. The efficacy of SU011248 is limited to patients carrying mutations in the exons 11 and 17.

#### *PTK787-ZK 222584*

PTK-ZK inhibits the tyrosine kinase activity of VEGFR1 and VEGFR2 and is given orally. Preclinical studies demonstrated activity through the inhibition of tumor vasculature, alone or in combination with chemotherapy or radiotherapy. Pre-irradiated vasculature may be more sensitive to PTK-ZK compared to vasculature not irradiated. Phase I studies have shown good PTK-ZK tolerance and efficacy in various tumors. The most commonly reported adverse events were nausea (59%), fatigue (41%), vomiting (35%), dizziness (29%) and headache (24%). The first study in 20 patients with relapsing glioblastoma yielded four stabilizations and one partial response in the 15 tested patients (Zakarija and Soff, 2005). Magnetic resonance imaging depicted a decrease in vascular permeability of tumors after intake of the drug that was correlated with clinical response. The second study enrolled 35 patients with advanced colorectal cancer and patients were treated every 14 days with oxaliplatin, 5-FU and folinic acid in combination with continuously escalated doses of PTK-ZK (500-2,000 mg day). No increased oxaliplatin/5-FU toxicity was seen and PTK-ZK was tolerated to doses of 1,500 mg day. In 28 patients, one complete response (4%), 14 partial (50%), nine SD (32%) and four progressive disease (14%) were obtained. For all patients, the median overall survival was 16.6 months (Arora and Scholar, 2005). Similar results were obtained when PTK-ZK was combined with irinotecan-5-FU-folinic. In the third study, 45 patients (37 evaluated) demonstrated the efficacy of PTK-ZK in metastatic renal cancer. Two patients experienced dose-limiting toxicity (grade 3 headache and grade 3 hypertension). Seven patients (19%) achieved a measurable response (1 partial and 6 minor) with a median time to progression of 5.5 months, 17 (46%) achieved SD and 5 (14%) experienced disease progression. Rapid disease progression (within 3 months) occurred in only 28% of the patients treated with at least 1,000 mg/day compared with an expected rate of 49.7% based on cytokine therapy in a similar patient population. One-year overall survival was 63.7% (95% CI = 41.9, 85.5%).

#### *Monoclonal Antibodies*

##### *Bevacizumab*

Bevacizumab is a humanized antibody designed to target VEGF and not its receptor like the other agents discussed in this review. This antibody has shown tumor growth inhibition in preclinical and early clinical studies with good tolerance. Phase I studies did highlight potential risk of hypertension or thromboembolism however its clinical efficacy has since been demonstrated. A phase III trial investigated bevacizumab (5 mg kg<sup>-1</sup> every two weeks) versus placebo in combination with irinotecan, 5-FU and folinic acid (FA) as first-line therapy for metastatic colorectal cancer in 925 randomized patients. Adding bevacizumab to chemotherapy resulted in increased median survival (20.3 months vs 15.6, p = 0.00003), progression-free survival (10.6 months vs 6.24, p < 0.00001), a higher response rate (45% vs 35%, p = 0.0029) and a longer duration of response (10.4 months vs 7.1, p = 0.0014) as

compared with chemotherapy plus placebo. Adding bevacizumab did not modify the toxicity of chemotherapy but only increased grade 3 hypertension (10.9 vs 2.3%), which was easily managed with oral medications (Hurwitz *et al.*, 2004). The inhibition of angiogenesis was demonstrated with a single infusion of bevacizumab that decreased tumor perfusion, vascular volume, microvascular density, interstitial fluid pressure and the number of viable, circulating mature and progenitor endothelial cells in patients with rectal carcinoma. A randomized, double blind, phase II trial was conducted comparing a placebo (40 patients) with bevacizumab at doses of 3 mg kg<sup>-1</sup> (37 patients) and 10 mg kg<sup>-1</sup> (39 patients), given every two weeks in 116 patients with metastatic renal cancer. There was a significant prolongation of the time-to-progression (TTP) in the high-dose antibody group as compared with the placebo group (hazard ratio, 2.55; p<0.001). The probability of having progression-free tumors for patients given the high-dose antibody, the low-dose antibody and the placebo was 64, 39 and 20%, respectively, at four months and 30, 14 and 5%, respectively, at eight months. There were no significant differences in overall survival between the groups but primary endpoints were TTP and the response rate (Yang *et al.*, 2003). Another phase II trial investigated the adjunction of bevacizumab to gemcitabine as first-line therapy in 21 patients with metastatic pancreatic cancer (Kindler *et al.*, 2005). Among evaluated patients, 6 PR (38%) and 7 SD (44%) were observed. Median survival was not reached and the estimated 1-year survival rate was 54% and TTP 5.5 months. Pretreatment VEGF levels ranged from 0 to 586 pg mL<sup>-1</sup> and were not correlated with response, TTP, or survival.

#### *IMC-1C11*

IMC-1C11, a chimeric IgG1 antibody against VEGFR2 and blocks ligand-receptor binding and inhibits phosphorylation. A phase I study in 14 patients with metastatic colorectal cancer resulted in prolonged stabilization but presence of anti-chimeric antibodies in 7 patients (2 had neutralizing antibodies) was detected (Posey *et al.*, 2003). Antibodies against VEGFR such as IMC-1C11 may have theoretical advantages compared to antibodies against soluble ligands that are frequently over produced leading to treatment resistance.

#### *Other Mechanisms to Inhibit the VEGF Pathway*

##### *Cellular Immunotherapy*

Using VEGFR-specific CD8<sup>+</sup> cytotoxic lymphocytes as opposed to antibodies is an original approach. A retroviral vector allows the transfection of VEGFR sequences in CD8<sup>+</sup> lymphocytes, which then become endowed with cytotoxic activity against VEGFR-expressing cells. These lymphocytes are able to inhibit tumor growth in mice *in vivo*. This inhibition is more powerful when administered in combination with the anti-angiogenic agent TNP-470 (Niederman *et al.*, 2002).

##### *Anti-FLT-1 Ribozyme*

Ribozymes are catalytic RNA molecules capable of cleaving mRNA at specific sequences. Anti-FLT-1 ribozyme is a nuclease-resistant synthetic molecule targeting VEGFR1 mRNA. In a phase I/II study enrolling 28 patients with refractory solid tumors, the ribozyme was subcutaneously administered daily. Tolerance was good apart from some reactions at the inoculation site (Weng *et al.*, 2005). Seventeen lasting 1-6 months and 2 minor responses were observed. Phase II studies testing ribozyme alone or in combination with chemotherapy are awaited.



#### *Aplidine*

Aplidine is a cyclodepsipeptide that seems to decrease VEGFR1 expression and to induce cell cycle arrest in the G1 phase. It has demonstrated promising activity *in vitro* against several human tumors. Aplidine is currently under clinical development in phase I studies and tumor responses have been reported particularly in leukemia patients (Jimeno *et al.*, 2004).

#### *Antisense Oligonucleotides*

Neutralizing antisense oligonucleotides for VEGF mRNA have been developed and exhibit an anti-angiogenic potential (Kamiyama *et al.*, 2002). A neutralizing antisense oligonucleotide for angiopoietin-1 was tested *in vitro* with HeLa tumor cell lines and showed a marked decrease in tumor growth.

#### *VEGF-Trap*

VEGF-trap is a fusion protein consisting of human VEGFR1 (flt-1) segments and VEGFR2 (KDR) extracellular domains fused to the Fc portion of human IgG1. It acts by binding to and inactivating circulating VEGF that found in tissues. VEGF-trap has great affinity for VEGF similar to that of the high-affinity receptor VEGFR1 ( $K_m = 1-5$  pM). This results in approximately 100-fold tighter binding than that achieved with anti-VEGF monoclonal antibodies, while retaining pharmacokinetic properties similar monoclonal antibodies. This compound could potentially achieve greater efficacy at lower doses compared to antibodies. Furthermore, in contrast to humanized monoclonal antibodies, it contains only human amino acid sequences. A dose-escalation phase I trial tested a single subcutaneous dose of VEGF-trap in patients with relapsed or refractory tumors, followed 4 weeks later by 6 weekly injections. Fourteen patients were treated at four dose levels (25, 50, 100 and 200  $\mu\text{g kg}^{-1}$ ). The VEGF-trap/VEGF complex in plasma had an apparent elimination half-life of approximately 17 days. No grade 3 or 4 toxicities were observed and no anti-VEGF-trap antibodies were detected. Grade 1 and 2 toxicities included reversible proteinuria, fatigue and constipation. Disease stabilization was obtained in patients with renal or colon cancer (Bergsland, 2004).

#### *Other Strategies to Target Tumor Vessels*

##### *COX-2 Inhibitors*

The production of VEGF and other angiogenic factors calls into service a cascade of intermediate signals, transcription and regulation factors and among the prostaglandins produced by cyclooxygenases (COX), the COX-2 isoform. COX-2 induction has been reported in numerous solid tumors (colon, prostate, lung, breast, pancreas, skin, head and neck) but not in corresponding normal tissues. COX-2 was detected in tumor cells, tumor vascularization and pre-existent adjacent capillaries. Tumors transplanted into COX-2 deficient mice showed that COX-2 is not only crucial for the production of VEGF, but that endogenous COX-2, produced by vascular endothelium or by fibroblasts, contributed markedly to the growth of transplanted tumor cells. The detection of COX-2 in neovasculature was described as a characteristic of epithelioma. In colon cancer, the number of cells expressing COX-2 was found to correlate with tumor stage, size and mediaurvival. Pre-clinical studies have demonstrated that anti-tumor activity of selective COX-2 inhibitors have a good safety profile. The anti-tumor properties of COX-2 inhibitors may be attributed to the inhibition of angiogenesis mediated by PGE and VEGF, however COX-2 may also play a role in the migration and survival of endothelial cells, vessel permeability and the suppression of immune responses. Collectively, these

events contribute to tumor growth and may eventually be potent therapeutic targets as assessed by several phase II studies in different tumor types. A recent trial tested the benefit of adding celecoxib (400 mg bid) to a chemotherapy cocktail containing irinotecan and capecitabine in the treatment of metastatic and unresectable colorectal cancer. Twenty-three patients were included (17 evaluated) and 9 PR (53%), 10 SD (56%) and 3 progressive disease (17%) were observed and celecoxib appeared to decrease the toxicity of capecitabine (Fayette *et al.*, 2005). A further study evaluated the combination of celecoxib (400 mg p.o. bid continuously) and paclitaxel (80 mg m<sup>-2</sup> i.v. weekly for six weeks followed by a two-week rest) in 27 patients (16 evaluated, mostly with adenocarcinomas) as second line therapy in non-small cell lung cancer (NSCLC) after failure of a first line with platinum compounds (Altorki *et al.*, 2003). Four PR (25%), 6 SD (37%) and 6 progressive disease (37%) were observed with acceptable toxicity. Another study evaluated the benefit afforded with celecoxib (400 mg · 2/d) combined with two preoperative cycles of paclitaxel and carboplatin in 29 patients (stages IB to IIIA). All patients completed preoperative chemotherapy and 26 completed preoperative celecoxib. The overall clinical response rate was 65% (48% PR, 17% complete responses). Twenty-eight patients were explored and underwent complete resection of their tumors. No complete pathological responses were observed but seven patients (24%) had minimal residual microscopic disease (Fayette *et al.*, 2005). Interestingly, it has recently been suggested that high COX-2 gene expression in completely resected NSCLC is correlated with poor prognosis (RR = 3.848; 95% CI, 1.500-9.874) and limited benefit with UFT-based adjuvant therapy. Indeed, in the setting of UFT postoperative adjuvant chemotherapy, patients with low COX-2 gene expression have a higher 5-year survival as compared to those with high COX-2 gene expression (93% vs 67%, p = 0.045) (Fayette *et al.*, 2005). A pilot study in 12 patients demonstrated the potential efficacy of celecoxib (200 mg · 2/day) in a biochemical relapse of prostate cancer (indicated by increased prostate-specific antigen (PSA) levels) after radiotherapy or radical prostatectomy. PSA levels were significantly modified in 8 patients after 3 months of treatment (declined in 5 and stabilized in 3). Of the remaining 4 patients, 3 had a marked decrease in their PSA doubling time. The short-term responses at 3 months also continued at 6 and 12 months (Pruthi *et al.*, 2004).

#### *Integrin Antagonists*

Vitaxin is a humanized monoclonal antibody against the  $\alpha V\beta 3$  integrin and is currently being tested in phase I trials where it has yielded PR or SD in sarcoma. EMD 121974, an  $\alpha V\beta 3$  and  $\alpha V\beta 5$  integrin antagonist, produced responses in melanoma and brain tumors in preclinical studies. A phase I trial resulted in good tolerance (Eskens *et al.*, 2003). Another humanized IgG1 (Medi-522) targeting  $\alpha V\beta 5$  integrins was tested in a phase I study which accrued 19 patients (13 evaluated). Tolerance was acceptable and 7 patients achieved prolonged SD with 2 lasting more than 9 months (McNeel *et al.*, 2005).

#### *Endogenous Angiogenesis Inhibitors*

Endostatin and angiostatin are peptides derived from natural proteins that have been shown to exhibit antiangiogenic properties. These agents have a short half-life and repeated, or even, continuous injections are required. Results are modest but prolonged SD was observed, particularly in patients with advanced neuroendocrine tumors who were enrolled in a phase II study. All the 41 patients (25 carcinoid and 16 pancreatic tumors) were initially treated with 60 mg m<sup>-2</sup> day<sup>-1</sup> of rhEndostatin, self-administered by subcutaneous injection in divided doses, at 12 h intervals. Minimal toxicity was

observed during this treatment. Doses were escalated if the therapeutic effect was insufficient but not for progressive disease. Twenty-three (62%) SD and 12 (32%) progressive disease were observed. The median TTP was 39 weeks and median survival was not reached (Eder *et al.*, 2002). Thrombospondin (TSP-1) is another natural angiogenesis inhibitor and ABT-510 is a substituted nanopeptide that mimics the anti-angiogenic activity of the endogenous protein. It was tested in a phase IB study that enrolled 36 patients (34 evaluated) with various tumors. Patients received daily subcutaneous escalating doses of ABT-510 with acceptable tolerance. One PR in a soft tissue sarcoma (STS) and 14 (41%) and 5 (15%) SD was observed in 8 and 16 weeks, respectively. Prolonged SD (>24 weeks) was seen in 1 patient with NSCLC and 1 with STS (Hoekstra *et al.*, 2005).

#### *Combretastatin A-4 Phosphate (CA4P)*

The phosphorylated pro-drug CA4P is a synthetic analogue of combretastatin, a tubulin-binding agent that selectively induces apoptosis in proliferating human umbilical vein endothelial cells *in vitro* and produced up to a 90% reduction in tumor blood flow in xenografted tumor models. Phase I studies showed its potential efficacy (Bilenker *et al.*, 2005; Stevenson *et al.*, 2003), but cardiotoxicity is the major adverse side effect.

#### *Raf-1 Pathway Inhibitors*

The  $\alpha$ V $\beta$ 3-integrin is preferentially expressed by the endothelium of neovessels and is a potential target for a gene therapy. A lipidic nanoparticle was synthesized and bound to an  $\alpha$ V $\beta$ 3-ligand. A gene could be coupled to this particle and delivered specifically to  $\alpha$ V $\beta$ 3-expressing cells. The selected gene was a mutated form of Raf that inhibits VEGF- and FGF-induced angiogenesis. Experiments in mice bearing melanoma or human colon tumor xenografts demonstrated very high anti-angiogenic activity, specifically mediated by the mutated Raf gene delivered to  $\alpha$ V $\beta$ 3-expressing endothelial cells, with fast and dramatic tumor regressions. Nanoparticles are much less immunogenic than viral vectors and thus allow repeated and prolonged treatment. A specific Raf-1 inhibitor has been developed: BAY43-9006. A phase I study showed good tolerance. A phase II study included 397 patients among which 112 with a renal cell carcinoma. Among them, 65 patients completed the treatment, 63 were evaluated with 25 (39%) PR and 16 (26%) SD (Clark *et al.*, 2005).

### **Conclusions**

Over the last few decades, rapid progress has been achieved in the understanding of tumor angiogenesis including signaling pathways and their regulation. This has enabled the development of numerous potentially interesting antineoplastic agents. However, further advances in dissecting the cascade of molecular events underlying cancer angiogenesis are necessary to fully exploit the therapeutic potential of VTAD. In particular, resistance to anti-angiogenic treatments has been observed to be due to mutations of the p53 protein (such is the case in 50% of human cancers) which lower tumor cell oxygen requirements and thus their dependence on neovascularization. The induction of bcl-2, a gene involved in resistance to apoptosis, has also been observed. Cross activation between the different signaling pathways must also be further elucidated. Studies in animals show that VEGFR and EGFR inhibitors can be combined to enhance their efficacy. One of the present challenges is to determine whether it is best to combine exclusively these new anti-angiogenic agents or to combine them with conventional cytotoxics so that the survival of patients can be increased significantly.

This challenge implies new models that preferably considers the cytostatic rather than the cytotoxic nature of anti-angiogenic agents, the possibility of prolonged therapy with these agents and the rationale for combining them with other cytotoxic therapies. Ongoing clinical trials are applying these concepts with the prospect of using these anti-angiogenic therapies in clinical practice.

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