



Michele Sassi. La Digue Island, c. 2001. Seychelles Islands, Indian Ocean. Photograph.

*Several strategies have been used to inhibit VEGF, including anti-VEGF monoclonal antibodies and agents that inhibit the VEGF receptor.*

# Clinical Experience With Angiogenesis Signaling Inhibitors: Focus on Vascular Endothelial Growth Factor (VEGF) Blockers

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**A**ngiogenesis is required for tumor growth and metastasis and, therefore, represents an exciting target for cancer treatment. Angiogenesis is a complex process that is tightly regulated by pro- and anti-angiogenic growth factors. Physiologic angiogenesis takes place during tissue growth and repair; during the female reproductive cycle, and during fetal development. Pathologic angiogenesis is characterized by either excessive (eg, cancer) or inadequate (eg, coronary artery disease) neovascularization. Angiogenesis occurs in a series of complex and interrelated steps that involve the release of pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF). VEGF regulates both vascular proliferation and permeability, and functions as an anti-apoptotic factor for newly formed blood vessels. The biological effects of VEGF are mediated by two receptors, VEGF-1 and VEGF-2, whose expression is largely limited to the vascular endothelium. VEGF is often expressed in tumors at substantially increased levels. It is expressed in response to hypoxia, oncogenes, and other cytokines, and its expression is associated with poor prognosis in several types of cancer. Several different strategies have been used to inhibit VEGF, including anti-VEGF monoclonal antibodies (eg, bevacizumab) and agents that inhibit the VEGF receptor (eg, SU5416). Both types of agents have tolerable side effects and have shown promise when evaluated in a wide range of tumor types. Angiogenesis, the role of VEGF in angiogenesis and malignancy, and strategies for cancer treatment with VEGF inhibitors are discussed.

## Introduction

Angiogenesis, the development of new blood vessels out of existing ones, is a fundamental requirement for new organ development and for differentiation during embryogenesis, wound healing, and reproductive

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functions in adults.<sup>1,4</sup> Compensatory angiogenesis is demonstrated in the formation of collateral blood vessels when there is oxygen or nutrient deprivation in normal tissues.<sup>5</sup> Angiogenesis also takes place in some pathologic conditions, such as rheumatoid arthritis, age-related macular degeneration, proliferative retinopathies, and psoriasis, as well as tumor growth and metastasis.<sup>1,2</sup>

The potential to treat tumors with anti-angiogenic therapy was first suggested more than 30 years ago.<sup>6</sup> Tumors require a supply of blood to nourish growth and facilitate metastasis. Therefore, neovascularization is essential for the growth of small tumors into larger ones that can continue to metastasize,<sup>6,7</sup> and angiogenesis represents an exciting potential target in cancer treatment.

## Angiogenesis

Angiogenesis is a complex process that is tightly regulated by pro- and anti-angiogenic growth factors

(Table 1). Some of these factors are highly specific for the endothelium (eg, vascular endothelial growth factor or VEGF), while others have a wide range of activities (eg, matrix metalloproteinases or MMPs).<sup>8</sup>

A variety of physiologic and pathologic stimuli can induce production of angiogenic growth factors. Physiologic angiogenesis takes place during tissue growth and repair, during the female reproductive cycle, and during fetal development. In some diseases, the body loses the ability to control angiogenesis and new blood vessel growth is either excessive (eg, cancer) or inadequate (eg, coronary artery disease).<sup>15</sup>

Angiogenesis occurs in a series of complex and interrelated steps (Fig 1). First, diseased or injured cells release pro-angiogenic growth factors, such as VEGF, FGF, or PDGF, into the surrounding tissue. Angiogenic growth factors are released in response to secreted proteins in the microenvironment (eg, epidermal growth factor, fibroblast growth factor, insulin-like growth factor, interleukins, platelet-derived growth factor),<sup>9-13</sup> hypoxia,<sup>14</sup> hypoglycemia,<sup>15</sup> mechanical stress (eg,

Table 1. — Pro-angiogenic and Anti-angiogenic Factors<sup>25</sup>

Pro-angiogenic Factors*	Anti-angiogenic Factors
Angiogenin	Angiostatin (plasminogen fragment)
Angiopoetin-1	Antiangiogenic antithrombin III
Del-1	Cartilage-derived inhibitor (CDI)
Fibroblast growth factor, acidic (aFGF)	CD59 complement fragment
Fibroblast growth factor, basic (bFGF)	Endostatin (collagen XVIII fragment)
Follistatin	Fibronectin fragment
Granulocyte colony-stimulating factor (G-CSF)	Gro-beta
Hepatocyte growth factor (HGF) <sup>†</sup>	Heparinases
Interleukin-8 (IL-8)	Heparin hexasaccharide fragment
Leptin	Human chorionic gonadotropin (hCG)
Midkine	Interferon alpha, beta, gamma
Placental growth factor (PlGF)	Interferon inducible protein (IP-10)
Platelet-derived endothelial cell growth factor (PD-ECGF)	Interleukin-12 (IL-12)
Platelet-derived growth factor-BB (PDGF-BB)	Kringle 5 (plasminogen fragment)
Pleiotrophin (PTN)	Tissue inhibitors of metalloproteinases (TIMPs)
Proliferin	2-Methoxyestradiol
Transforming growth factor-alpha (TGF-alpha)	Placental ribonuclease inhibitor
Transforming growth factor-beta (TGF-beta)	Plasminogen activator inhibitor
Tumor necrosis factor-alpha (TNF- $\alpha$ )	Platelet factor 4 (PF4)
Vascular endothelial growth factor (VEGF) <sup>‡</sup>	Prolactin 16kD fragment
	Retinoids
	Tetrahydrocortisol-S
	Thrombospondin-1 (TSP-1)
	Transforming growth factor beta (TGF- $\beta$ )
	Vasculostatin
	Vasostatin (calreticulin fragment)

\* Factors known to activate endothelial cell growth and movement.  
<sup>†</sup> Also known as scatter factor (SF).  
<sup>‡</sup> Also known as vascular permeability factor (VPF).

increased pressure caused by proliferating tumor cells),<sup>16</sup> release of inflammatory proteins (eg, cyclooxygenase-2, prostaglandins, mast cell activation),<sup>17-19</sup> and genetic alterations.<sup>20,21</sup> The released growth factors bind to, thereby activating, endothelial cells that form the walls of nearby blood vessels.<sup>22,23</sup>

Activated endothelial cells signal their nucleus to produce enzymes, such as MMPs. These enzymes break down the extracellular matrix of the blood vessel, allowing endothelial cells to invade the matrix and to divide in response to tumor-derived growth factors. The proliferating endothelial cells migrate through the holes made by the enzymes toward the growth factor stimulus. Adhesion molecules or integrins mediate the migration of the new endothelial cells toward the growth factor stimulus and additional enzymes are released to dissolve the surrounding tissue. The adhesion receptor integrin  $\alpha_v\beta_3$ , present on the surface of activated endothelial cells, is required for the differentiation, maturation, and survival of blood vessels.<sup>22-24</sup>

Strings of new endothelial cells organize into hollow tubes, thus creating new blood vessels, and individual blood vessels connect to form vessel loops or networks that allow blood to circulate. Structural support is provided to the new blood vessels by pericytes. The vessels are then ready to carry blood to the tissue that initially released the pro-angiogenic growth factors.<sup>22,23,25</sup>

## The Role of Vascular Endothelial Growth Factor (VEGF) in Angiogenesis

VEGF regulates both vascular proliferation and permeability. Also known as vascular permeability factor (VPF), it is unique among pro-angiogenic factors because of its specificity for vascular endothelium and potency.<sup>26</sup> It also functions as an anti-apoptotic factor for endothelial cells in newly formed vessels.<sup>27</sup> VEGF is expressed in tumor cells, macrophages, T cells, smooth muscle cells, kidney cells, mesangial cells, keratinocytes, astrocytes, and osteoblasts.<sup>28</sup>

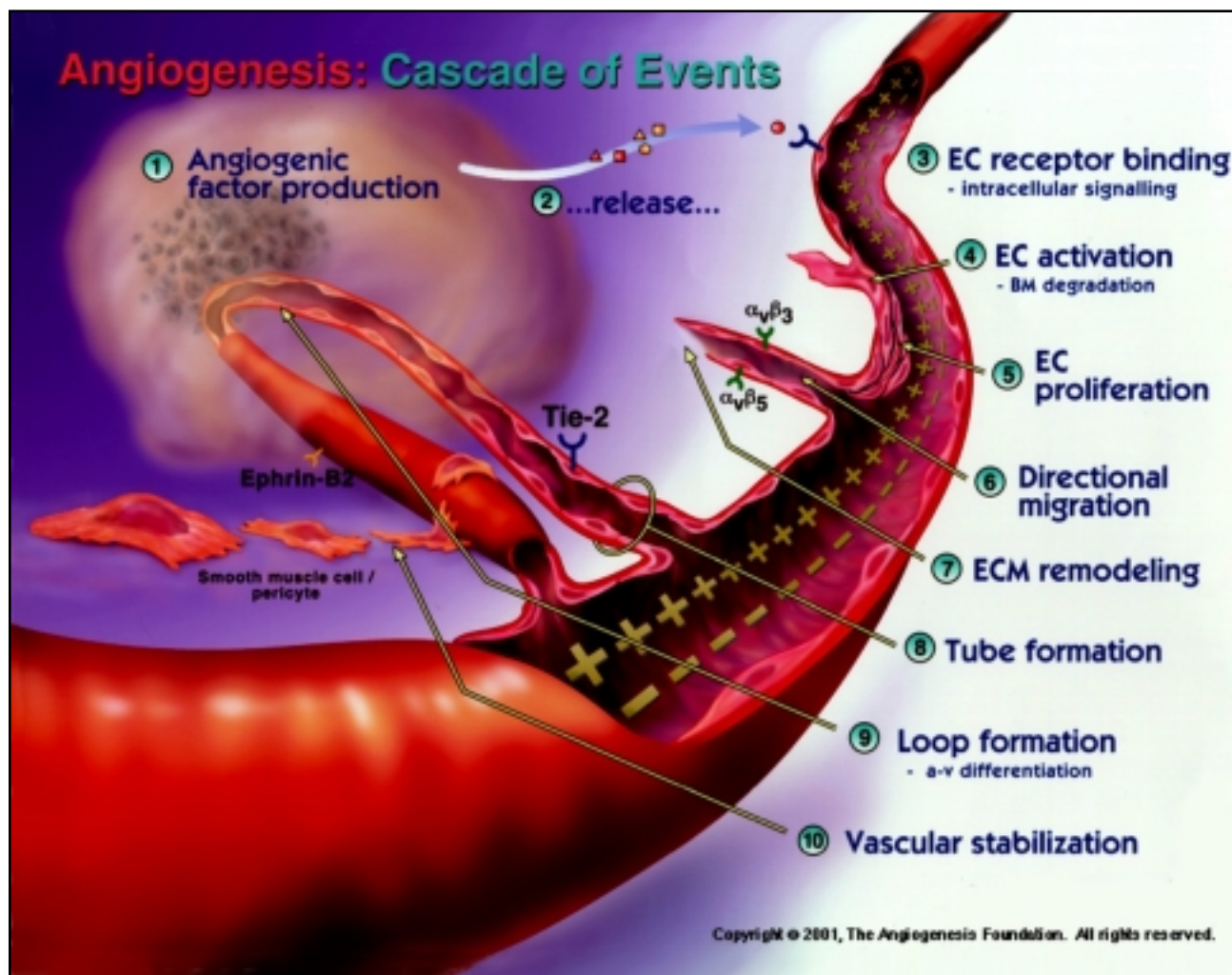


Fig 1. — Angiogenesis: the formation of new blood vessels. Source: The Angiogenesis Foundation (www.angio.org).

There are currently six known members of the VEGF family: VEGF, placental growth factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E.<sup>29</sup> VEGF is a diffusible endothelial cell-specific mitogen and pro-angiogenic factor that increases vascular permeability. VEGF mitogenic activity is demonstrated in arteries, veins, and lymphatics, but not to an appreciable extent in other cell types.<sup>30</sup>

Survival of endothelial cells in newly formed vessels is VEGF-dependent.<sup>27,31,32</sup> Knocking out just one allele of the VEGF gene in mice leads to embryonic death, suggesting an essential role for VEGF in vascular development.<sup>31</sup>

It has been proposed that an increase in vascular permeability is essential to angiogenesis in tumors and wounds and that a major function of VEGF is the induction of plasma protein leakage, resulting in the formation of an extravascular fibrin gel. This fibrin gel serves as the substrate for endothelial and tumor cell growth.<sup>33</sup> VEGF increases permeability by inducing pores or fenestrations in the endothelium.<sup>26,34</sup>

Several mechanisms may regulate VEGF gene expression, the most important of which may be hypoxia. Other factors shown to up-regulate VEGF mRNA expression include cytokines, such as epidermal growth factor, TGF- $\beta$ , or keratinocyte growth factor, cell differentiation, and oncogenes.<sup>30,35</sup>

The biological effects of VEGF are mediated by two receptor tyrosine kinases (RTKs), VEGFR-1 (Flt-1 or *fms*-like-tyrosine kinase) and VEGFR-2 (Flk-1/KDR).<sup>28</sup> The expression of these receptors is largely restricted to the

vascular endothelium<sup>30,36</sup> and it is assumed, but not proven, that all of the effects of VEGF on vascular endothelium are mediated by these receptors. VEGFR-2 is thought to be the dominant signal transduction pathway regulating angiogenesis.<sup>26</sup> When VEGF binds to its receptor, the pro-angiogenic signal is transmitted by the RTK to downstream proteins, initiating a signal cascade (Fig 2).<sup>26</sup>

## The Role of VEGF in Malignancy

Tumors require angiogenesis to grow beyond 1 to 2 mm<sup>3</sup> in size<sup>1</sup> and to facilitate metastasis.<sup>37</sup> VEGF plays a central role in tumor angiogenesis; it is expressed in most tumors, often at substantially increased levels.<sup>38</sup> VEGF expression in tumor cells is stimulated by hypoxia<sup>14</sup> and by oncogenes such as *ras* or by inactivation of tumor suppressor genes, and by other cytokines.<sup>30,35,39</sup>

There are two phases in tumor neovascularization — the pre-vascular phase (also called the “angiogenic switch”) and the vascular phase.<sup>40</sup> Tumor cells that undergo the phenotypic switch are able to induce phenotypic changes in endothelial cells, leading to angiogenesis.<sup>41</sup> Tumor cells and infiltrating cells such as macrophages and fibroblasts activate the endothelial cells, thus initiating angiogenesis by expressing factors such as VEGF and bFGF. Once neovascularization occurs, the tumor experiences rapid growth and an increased metastatic potential.<sup>39,42,43</sup>

VEGF mRNA is markedly up-regulated in most human tumors. Whereas VEGF mRNA is up-regulated in tumor cells but not in endothelial cells, Flt-1 mRNA and KDR mRNA are up-regulated in the endothelial cells associated with the tumor.<sup>30</sup>

There is a correlation between tumors with higher densities of blood vessels and metastasis and poorer clinical outcome. Expression of VEGF is associated with tumor growth, angiogenesis, and metastasis and anti-VEGF antibodies inhibit tumor growth in vivo in a nude mouse model.<sup>44</sup> In an animal model of mammary cancer, overexpression of VEGF and the Flk-1 receptor are correlated with high Ki-67 reactivity, suggesting that VEGF may have an autocrine regulatory role mediated through Flk-1 in breast cancer.<sup>45</sup>

VEGF levels appear to have prognostic significance in human tumors.<sup>44,46-58</sup> Expression of VEGF is associated with poor prognosis in acute myeloid leukemia,<sup>59</sup> breast cancer,<sup>60-62</sup> colon cancer,<sup>56,63,64</sup> hepatocellular carcinoma,<sup>65</sup>

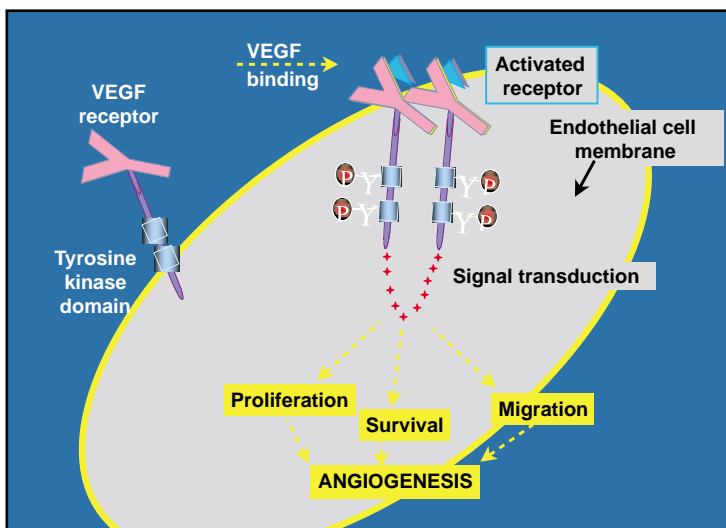


Fig 2. — The VEGF pathway. This diagram shows a cross-sectional view of an endothelial cell and depicts the VEGF pathway in angiogenesis. Binding of VEGF to its receptor VEGFR-2 results in dimerization and activation of the receptor molecule. Activation of the receptor molecule initiates a cascade of cellular events, leading to proliferation, migration, and differentiation of endothelial cells.

non-small cell lung cancer,<sup>66,67</sup> and ovarian cancer.<sup>68</sup> In a study conducted in patients with primary lung cancer, median survival was 151 months in patients with low VEGF levels compared with 8 months in patients with high VEGF levels.<sup>69</sup> In immunohistochemical analysis of archival specimens of human colon cancers and adenomas, expression of VEGF and KDR was higher in metastatic compared with nonmetastatic neoplasms and was directly correlated with extent of proliferation and neovascularization.<sup>56</sup>

VEGF expression in tumors and cytosols has been correlated with outcome in women with breast cancer<sup>43,61,62,70-74</sup> and may be useful in making treatment decisions and in assessing treatment response. In women with node-negative breast cancer, there is a significant association between VEGF expression, estrogen receptor status, progesterone receptor status, and tumor size. Patients with a higher VEGF expression have significantly reduced relapse-free survival and overall survival.<sup>61,62,72</sup> In women with node-positive breast cancer, VEGF expression also can predict relapse-free survival and overall survival.<sup>70</sup> Using multivariate analysis, VEGF expression was shown to be an independent predictor of overall survival in women with primary breast cancer. Patients with p53 positivity and high VEGF expression had an increased relative risk for poorer overall survival of 2.7 compared with 1.7 for patients with only one of these risk factors.<sup>71</sup>

A correlation has been demonstrated between VEGF levels and menopausal status. In normal breast tissue, VEGF levels are significantly higher in premenopausal women compared with postmenopausal women and are inversely correlated with age. This suggests that the ovarian steroids (ie, estrogens and progestins) may be involved in VEGF regulation. However, the hormonal regulation of VEGF seems to disappear in women with breast cancer; there is no correlation between menopausal status and VEGF levels in breast cancer tissue.<sup>75</sup>

## Cancer Treatment Through VEGF Inhibition

VEGF and its receptors are good targets for cancer therapy because VEGF receptors are highly specific for VEGF and are expressed in increased numbers primarily during periods of tumor growth.<sup>37</sup> Several different strategies have been used to inhibit VEGF, including anti-VEGF monoclonal antibodies,<sup>76,77</sup> coupling a toxin to VEGF,<sup>78</sup> soluble VEGF receptors,<sup>79,80</sup> peptides that interfere with VEGF binding,<sup>81</sup> and agents that block VEGF receptor signaling.<sup>82-86</sup>

VEGF inhibitors differ from traditional cytotoxic chemotherapy in many ways; therefore, the strategies used with the cytotoxic agents may not apply to VEGF inhibitors. While traditional chemotherapy targets cancer cells, VEGF-inhibitors target normal endothelial cells. Regulatory agencies insist on objective measurements of activity. That approach may not be appropriate for anti-angiogenic agents such as VEGF inhibitors, because they do not kill tumor cells but instead hold them in check. Cytostatic agents such as VEGF inhibitors may be more appropriately evaluated based on their effect on time to tumor progression (TTP) or survival. Moreover, cytostatic agents may need to be dosed for chronic administration, not with the intent of achieving the maximum tolerated dose.<sup>87</sup>

Angiogenesis inhibitors may be most effective when combined with traditional cytotoxic chemotherapy. There are several reasons to support this hypothesis. The cellular target for the angiogenesis inhibitors differs from that of cytotoxic agents; therefore, combined use should lead to reduced tumor burden without overlap in patterns of resistance. In fact, since angiogenesis inhibitors target normal, genetically stable endothelial cells, resistance to these agents may not develop.<sup>88,89</sup> The side effect profiles of cytostatic and cytotoxic agents can be very different. For example, angiogenesis inhibitors often do not cause myelosuppression and, therefore, should allow for administration of the maximal dose of the cytotoxic agent without fear of additive toxicity.<sup>87</sup> There is extensive experience with combination therapy in many animal models.<sup>90</sup>

The development of ascites and pleural effusions (PEs) is associated with various malignancies. Although these processes have traditionally been thought to be a result of insufficient lymph drainage,<sup>91</sup> there is evidence to suggest that VEGF may play a role. Ascites and PE fluid contain very high levels of biologically active VEGF,<sup>92-94</sup> suggesting a role for VEGF inhibitors in the treatment of these conditions. Whether systemic administration of the angiogenesis inhibitor is sufficient or direct administration into the affected cavity is required will be the subject of future clinical investigation.

## SU5416

SU5416 (Semaxanib; SUGEN, Inc, South San Francisco, Calif) is a small molecule inhibitor of VEGFR-2, Flt-1, and c-kit that has provided promising clinical results.<sup>85</sup> SU5416 is a competitive inhibitor, with regard to ATP, inhibiting the activity of Flk-1/KDR receptor kinase.<sup>84</sup> It also exhibits some activity against platelet-derived growth factor (PDGF) receptor, Flt-1, Flt-4, and c-kit.<sup>95</sup>

In *in vitro* studies, SU5416 exerted a potent and rapid antiproliferative effect on endothelial cells without directly affecting tumor cell growth in culture. When administered to mice, it inhibited the growth of tumor cells in a variety of tumor models.<sup>84</sup> The direct effect of SU5416 on tumor angiogenesis was assessed by implanting C6 rat glioma cells into the dorsal skin-fold chamber of athymic mice who were then treated with SU5416. The newly formed microvasculature within (intratumoral) and around (peritumoral) the tumor was evaluated. Increased angiogenic activity and vascular density were noted in peritumoral cells compared with intratumoral cells. By 22 days post-implantation, there was up to a 92% decrease in tumor growth. A reduction in the total and functional vascular density and vascular leakage was also reported.<sup>85</sup> Tumor growth inhibition has been found to be similar regardless of whether treatment with SU5416 is started one day after implantation or after the tumor has grown to measurable size (100 mm<sup>3</sup>).<sup>96,97</sup> In mice, SU5416 has been shown to reverse tumor resistance to radiotherapy<sup>98</sup> and in a mouse metastasis model, to inhibit the growth of metastases.<sup>99</sup>

In human tumor xenograft models, SU5416 administration once or twice weekly was as effective as daily administration, despite the fact that its half-life is only 30 minutes. Three-hour exposure to 5  $\mu$ M SU5416, which approximately mimics plasma levels obtained in patients, produces activity for at least 72 hours *in vitro*. The durability of response is attributed to the concentration of SU5416 in cells, allowing for long-term inhibition of VEGF-dependent phosphorylation of Flk-1/KDR and subsequent downstream signaling.<sup>100</sup>

In a phase I study, SU5416 doses were administered twice weekly based on these preclinical observations; doses ranged from 4.4 to 190 mg/m<sup>2</sup>. A total of 69 patients with advanced malignancies were treated. The dose-limiting toxicities were reversible grade 3 headaches, nausea, and grade IV vomiting, lasting about 1-2 days; high doses of narcotics and antiemetics were ineffective in controlling these symptoms.<sup>83,101</sup> Based on data from this phase I study, the maximum tolerated dose is 145 mg/m<sup>2</sup> intravenously twice weekly and is associated with tolerable side effects, including headache, nausea, phlebitis, urine color change, diarrhea, and vomiting.<sup>102</sup>

SU5416 also has been tested in patients with AIDS-related Kaposi's sarcoma. Among 18 evaluable patients with stable or progressive disease enrolled in a dose-escalating study, 61% had improvement (ie, flattening, shrinkage, or dissolution of lesions, reduction or dissolution of edema), 22% had stable disease, and 17% had progressive disease after twice weekly intravenous

SU5416 for up to 4 cycles of therapy (29 days per cycle). Responding patients reported reduced pain and increased mobility.<sup>103</sup>

Clinical trials using SU5416 alone or in combination with cytotoxic chemotherapy are being conducted in a variety of tumor types.

## Bevacizumab

The RhuMab-VEGF bevacizumab (Avastin, Genentech, South San Francisco, Calif) also shows promise in cancer treatment. In *in vivo* and *in vitro* models, RhuMab-VEGF has been shown to reduce VEGF levels.<sup>104</sup> Selective inhibition of VEGF receptor 2 (KDR/Flk-1) using a RhuMab-VEGF (2C3) blocks tumor growth in mice.<sup>105</sup>

In phase I studies in patients with advanced cancer, bevacizumab has been safely administered alone and in combination with chemotherapy.<sup>76,106</sup> Bevacizumab efficacy was subsequently evaluated in a phase II study enrolling 104 patients with previously untreated metastatic colon cancer. Patients were randomized to treatment with standard chemotherapy alone (5-fluorouracil 500 mg/m<sup>2</sup> plus leucovorin 500 mg/m<sup>2</sup> weekly for the first 6 weeks of an 8-week cycle), the same chemotherapy in combination with a low dose of bevacizumab (5 mg/kg every 2 weeks), or the same chemotherapy in combination with a high dose of bevacizumab (10 mg/kg every 2 weeks).<sup>107</sup>

In this phase II study, the response rate was 40% in the low-dose group, 24% in the high-dose group, and 17% in the chemotherapy-alone group. Time to disease progression was 9.0 months in the low-dose group, 7.2 months in the high-dose group, and 5.2 months in the chemotherapy-alone group. Median survival was not yet reached in the low-dose group at a follow-up of 17.3 months, but was 16.1 months in the high-dose group and 13.6 months in the chemotherapy only group. Treatment was generally well tolerated; fever, chills, headache, hypertension, infection, rash, and nosebleeds were more common in patients treated with the MAb.<sup>107</sup> Others have also reported promising results with RhuMAbs to VEGF.<sup>106,108,109</sup>

## Discussion and Conclusions

The pro-angiogenic factor VEGF and its receptors are expressed in a wide range of human tumors. Angiogenesis is involved in nearly every stage of cancer, from the first stage of cancer formation to the final stage of distant metastasis.

Angiogenesis involves a series of steps, including endothelial cell proliferation, differentiation, migration, and organization to form tubules.<sup>26</sup> Because of this stepwise process, anti-angiogenic therapy can be developed against any of several steps in the process. Angiogenesis is a rate-limiting factor in tumor growth and metastasis and increased vascularization has been directly linked to poor prognosis.<sup>44</sup> Moreover, there is compelling evidence that circulating VEGF levels are of prognostic significance in a variety of tumor types.<sup>44,46-58,61,62,69,72</sup>

VEGF inhibition can be an attractive therapeutic strategy because it is highly specific and may be less toxic than cytotoxic therapy. VEGF inhibitors offer a means to control a heterogeneous tumor population by influencing a relatively homogeneous endothelial population. Theoretically, VEGF inhibitors should control tumor growth independent of specific tumor details and induce a dormant state in which pro-angiogenic and anti-angiogenic factors come back into balance and tumor growth is controlled. Because anti-angiogenesis agents will likely stabilize tumor growth but not reduce tumor burden, they may be best suited for long-term therapy.

The best method for selection of patients for VEGF inhibitor administration and for assessment of drug efficacy must still be developed. The most common measure of tumor angiogenesis is microvessel density, but this is not a convenient treatment end point because measurement would require serial biopsies and the results are, at best, subjective. Surrogate markers of angiogenesis under study include VEGF or bFGF levels, which can be measured in the blood or urine.<sup>23</sup>

An important consideration in the use of VEGF inhibitors is whether protective functions of the cytokine might be inhibited by its use, resulting in delayed toxicity. Autocrine VEGF production serves a protective role against endothelial cell damage, including that caused by cytotoxic agents.<sup>110</sup> Anti-angiogenesis agents may affect wound healing, physiologic reproductive angiogenesis in women, and prenatal and neonatal growth. However, the use of highly specific agents may permit the avoidance of effects on physiologic angiogenesis<sup>88</sup> since there is probably redundancy in many of the physiologic angiogenesis processes.<sup>111</sup> However, this same redundancy could render a VEGF inhibitor ineffective, hence the need to observe patterns of failure as well as patterns of success.

Trials are currently underway to evaluate several anti-angiogenesis agents, including SU5416, bevacizumab, TNP-470, thalidomide, SU6668, ZD4190, ZD6474, and PTK787. SU5416 and bevacizumab are among the

first VEGF inhibitors to be tested in clinical trials. Initial results are promising but need to be validated in larger studies. Short-term toxicities appear to be tolerable, and there is no evidence of long-term toxicities based on limited use. Ongoing clinical trials are assessing efficacy alone and in combination with cytotoxic agents in various advanced malignancies.

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