The Emerging Role of Angiogenesis Inhibitors in Hematologic Malignancies

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Angiogenesis is an important component of the pathogenesis of hematologic malignancies. A negative prognostic implication of increased angiogenesis has been established for acute and chronic myeloid and lymphocytic leukemias, myeloproliferative diseases, multiple myeloma, non-Hodgkin’s lymphoma (NHL), and hairy cell leukemia. An association between the return of increased marrow vascularity to normal levels and durability of response has been established in some of these diseases.

Data on the significant role of angiogenesis in the hematologic disorders—including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), the myelodysplastic syndromes (MDS), non-Hodgkin’s lymphoma (NHL), hairy cell leukemia, multiple myeloma, and agnogenic myeloid metaplasia—is growing in an exponential manner (Table 1). [1-26] Bone marrow microvessel density (MVD) is significantly greater in patients with advanced MDS (ie, refractory anemia with excess blasts [RAEB] or RAEB in transformation [RAEB-t]) compared with normal individuals. Patients with AML also have a greater bone marrow MVD, [10] and successful induction chemotherapy has resulted in a significant decrease in bone marrow MVD in these patients. [3] In children with ALL, a complete remission (CR) associated with a return to normal bone marrow MVD is more durable than a CR associated with a persistent increase in MVD. [4] In patients with AML, ALL, and MDS, in addition to the relative increase in MVD, there is a shift from the normal bone marrow predominance of straight, nonbranching microvessels to vessels with a complex, arborizing architecture. In patients with multiple myeloma, increased MVD has been directly correlated with the plasma cell labeling index. [27]

The Proper Angiogenesis Modulator to Target

As summarized in Table 2, there are numerous molecules to target within the angiogenesis cascade. However, one factor, vascular endothelial growth factor (VEGF), has emerged as the prime target in treating hematologic malignancies. Vascular endothelial growth factor is pivotal in the angiogenic process and regulates several endothelial cell functions, including mitogenesis, permeability, vascular tone, and the production of vasoactive molecules. [28] Transcription of the VEGF gene (located on the short arm of chromosome 6 at 6p21.3) is physiologically regulated by hypoxia. Production of VEGF falls under the control of alternate mRNA splicing and proteolytic processing. Alternate splicing is responsible for the production of the isoforms VEGF 189, VEGF 165, VEGF 121, and VEGF 205. [16]

The activity of VEGF is stimulated by three receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flik-1), and VEGFR-3 (Flt-4). Primarily expressed on endothelial cells and monocytes, VEGFR-1 mediates cell motility. [28] The proliferative and mitogenic activities of VEGF and increases in vascular permeability are mediated primarily via VEGFR-2. [29] Finally, VEGFR-3, homologous to the neuropilin-1-receptor, is involved in lymphoangiogenesis. [30]

VEGF Activity in Hematologic Malignancies

Significantly elevated levels of VEGF are observed in a variety of hematologic malignancies (Table 3). In a study of 417 patients, a number of patients with AML (n = 115) and advanced MDS (n = 40) had similar elevated plasma levels of VEGF. [31] In stored serum samples, increasing levels of cellular VEGF had a positive correlation with a lower CR rate, shorter CR duration, and shorter overall and disease-free survival times. [9] These data are in contrast to those reported for the receptor tyrosine kinase Tie-1, in which we used Western blot analysis to confirm and radioimmunoassay to quantify Tie-1 protein expression in bone marrow samples obtained from untreated patients with AML (n =
66) or MDS (n = 29). Tie-1 protein levels were elevated in all disease specimens and were significantly higher in patients with AML than in patients with MDS. However, Tie-1 levels did not correlate with CR or survival duration in patients with either AML or MDS.

This contrast with respect to VEGF results is particularly interesting when one considers the relative roles of Tie-1 and VEGF in marrow angiogenesis. Tie-1 is expressed on both vascular endothelial cells and immature hematopoietic cells, as is VEGF. Although Tie-1-deficient mice with a targeted insertional mutation in their germ line have defects in endothelial cell integrity, resulting in edema and hemorrhage, data indicate that Tie-1 is not critical for either bone marrow stem cell engraftment or self-renewal. Interactions between proangiogenic molecules and marrow stromal elements may also contribute to AML growth (Figure 1). For example, incubation of human umbilical vein endothelial cells with VEGF results in increased secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF), an AML blast growth factor, by the endothelium.

We have recently evaluated the clinical significance of VEGFR-1 and VEGFR-2 protein levels in patients with untreated AML and MDS to identify any relationship between these receptors and VEGF levels in each disease. Levels of VEGFR-1 were significantly higher in AML than in MDS patients, and VEGFR-2 levels were equivalent. There was no correlation between VEGFR levels and survival. Levels of VEGF were significantly higher in MDS than in AML patients, and levels correlated with poorer survival in MDS patients. There was a significant correlation between VEGF and VEGFR-2 levels in both AML and MDS patients, but not between VEGF and VEGFR-1 levels in either disease. These data suggest that VEGF expression, not VEGFR expression, is of prognostic relevance in AML and MDS. The significance of the differential expression of VEGFR in AML and MDS is unclear.

**Chronic Lymphocytic Leukemia**

Intracellular levels of VEGF have also been correlated with prognosis in patients with CLL. In an analysis of samples collected from patients (n = 225) with B-cell CLL, the median intracellular VEGF level was 7.26 times higher than that of normal peripheral blood mononuclear cells. Patients with lower levels of VEGF protein showed a trend toward a relatively reduced rate of survival. In a subgroup of patients with Rai stages 0 to II, Binet stage A and B disease, and good prognostic features (eg, beta-2-microglobulin of 2.8 mg/dL or less), lower levels of VEGF were also associated with shorter survival times. However, no overall correlation among VEGF, disease stage, and beta-2-microglobulin levels was demonstrated in the cohort.

In a separate report, there was an indirect correlation between elevations in serum VEGF and the duration of progression-free survival (PFS) in patients with Binet stage A disease. Molica et al combined the patients’ Rai classification with serum VEGF levels, and identified two groups with different PFS within stages I and II. Patients with Rai stage I to II with elevated VEGF levels had very short PFS (median, 9.5 months) compared with patients with Rai stage I to II with low VEGF levels, who had longer PFS (median not reached at 15 months).

In a recent examination of the role of VEGFR-2 in CLL, patients with elevated VEGFR-2 levels had greater elevations in lymphocyte counts, severe anemia, elevated serum beta-2-microglobulin levels, and advanced-stage disease. Elevated VEGFR-2 levels were associated with reduced survival. Therefore, further studies on the interactive effects of VEGF and VEGFR-2 on CLL proliferation are warranted. An analysis of Flt-1 and Tie-1 protein levels in this same cohort of CLL patients also showed that these protein levels correlate with white blood cell counts and absolute lymphocyte counts. Flt-1 protein levels correlated with levels of cellular VEGF, whereas levels of Tie-1 did not. Furthermore, neither correlated with survival in the overall cohort; however, higher levels of Tie-1, but not Flt-1, correlated with reduced survival.

Although high levels of both VEGF and basic fibroblast growth factor (bFGF) have been documented in patients with CLL, bone marrow MVD is not increased in most of these patients. As observed in AML with VEGF levels, aside from any proangiogenic role, VEGF and bFGF have significant, independent roles in the pathophysiology of CLL.

**Lymphomas**
Elevated levels of VEGF are also associated with an adverse prognosis in patients with NHL.[25] However, the highest prognostic power was observed when VEGF and serum bFGF levels were examined in combination. The risk of death in patients whose VEGF and bFGF levels were both within the highest quartiles was greater than in other patients, independent of the prognostic variables in the International Prognostic Index.[25] In collaboration with the Omaha group, we have recently analyzed the clinical significance of serum levels of VEGF, bFGF, hepatocyte growth factor (HGF), and angiogenin in untreated patients with NHL or Hodgkin’s disease (HD). In patients with HD or NHL, VEGF and HGF levels were significantly increased (personal communication, M. Albitar, 2002). In contrast, angiogenin levels were significantly decreased in patients with either NHL or HD.

As reported by Salven et al, higher levels of VEGF and bFGF in NHL patients correlated with more advanced disease stage and with higher lactate dehydrogenase levels.[25] In addition, elevated levels of VEGF correlated with shorter survival in patients with HD. Elevated baseline VEGF levels tended to return to normal in patients with NHL who responded to therapy, and no significant changes in the levels of other factors were observed. In posttherapy samples collected from patients with HD, HGF and bFGF returned to normal levels, whereas VEGF levels remained elevated. Patients with NHL with higher posttherapy VEGF levels had relatively reduced survival and VEGF levels after therapy remained predictive of survival in patients with HD. Angiogenic factors appear to have distinct roles in the biology of HD and NHL, and a better appreciation of these differences may aid in the diagnosis of some patients.

**Multiple Myeloma**

In patients with multiple myeloma, levels of VEGF, bFGF, and HGF parallel disease activity, and VEGF levels correlate with features of aggressive disease, including levels of serum C-reactive protein and beta-2-microglobulin I.[7] Multiple myeloma patients have significantly higher levels of VEGF in bone marrow than in peripheral blood}[malignant plasma cells have been shown to express and secrete VEGF. Although marrow MVD parallels disease activity in MM, and it is thus reasonable to postulate that VEGF is acting in an autocrine fashion, multiple myeloma cells have a low level of VEGFR expression. Thus, VEGF may act in a paracrine fashion in multiple myeloma[both by its interactions with interleukin-6, a known myeloma growth factor, and with bFGF. Levels of bFGF have been reported to correlate with response rates to thalidomide (Thalomid) treatment in multiple myeloma patients.[35] Because HGF is overproduced in multiple myeloma and malignant cells express the HGF receptor c-met, this may be the basis for another autocrine loop in multiple myeloma.[36]

**Cyclooxygenase and Hematologic Malignancies**

Cyclooxygenase (COX, prostaglandin G/H synthase) is a membrane-bound enzyme responsible for the oxidation of arachidonic acid to prostaglandins, and this enzyme has two isoforms, COX-1 and COX-2.[37] Cyclooxygenase-1 is constitutively expressed and regulates homeostatic functions including vascular hemostasis and gastroprotection. Cyclooxygenase-2 is inducible by mediators such as growth factors, cytokines, and endotoxins. Nonsteroidal anti-inflammatory drugs produce their therapeutic effects through inhibition of COX prostaglandin formation. The expression of proangiogenic factors, including VEGF, bFGF, transforming growth factor-beta, and interleukin-6, is stimulated by prostaglandins.[38] Recent efforts have focused on the development of selective inhibitors of COX-2, which avoid the adverse effects associated with COX-1 inhibition. Many human solid tumors overexpress COX-2 but not COX-1, and gene-knockout and transfection experiments demonstrate a central role of COX-2 in experimental tumorigenesis.[39] Elevated COX-2 levels have adverse prognostic significance and/or are associated with biologic features favoring metastatic spread, recurrence, or local invasiveness in a number of human solid tumors.

Cyclooxygenase inhibitors have been shown to decrease the rate of development of human colorectal tumors.[40] Cyclooxygenase-2 has also been shown to affect the relationship between malignant cells and adjacent stroma. Abnormalities in this relationship abound in the bone marrow of patients with hematologic malignancies. Cyclooxygenase-2 has also been shown to regulate differential expression of apoptosis-related genes, thus favoring cell survival and potentially mediating resistance to anticancer therapy.

**COX-2 and UPP Inhibition**
Perturbation of the apoptotic cascade is well documented in leukemia, multiple myeloma, and NHL, especially in terms of abnormal function of members of the Bcl-2/Bax family. Selective inhibition of COX-2 has been shown to enhance cytotoxin-induced apoptosis in cancer models. We have recently observed that COX-2 levels are significantly elevated in patients with CML. No significant differences were evident in the levels of COX-2 expression in patients with either early or late chronic phase CML. The elevation of COX-2 was prognostically adverse in patients with chronic phase CML and was a more important independent prognostic factor than the established CML staging systems for these patients. Cyclooxygenase-2 inhibition may be an important area of investigation in hematologic malignancies, particularly in CML, AML, and MDS.

Another important pathway under investigation to indirectly suppress VEGF involves inhibition of the ubiquitin-proteasome pathway (UPP). The 26S proteasome, an adenosine triphosphate-dependent multicatalytic protease, is responsible for the UPP breakdown of many intracellular proteins in eukaryotic cells. The 26S proteasome (a core 20S particle bound to two regulatory 19S particles) lyses misfolded, oxidized, or damaged ubiquinated proteins, including those that regulate cell cycle and trafficking, transcription factor activation, and apoptosis. Cell mitosis is dependent on the orderly degradation of key regulatory proteins by the UPP, including p53, cyclins, and the cyclin-dependent kinase inhibitors p21 and p27 K1p1.

PS-341 (aka LDP-341 or MLN-341) is a potent, sensitive, selective, and reversible small-molecule proteasome inhibitor that binds tightly to the enzyme's active sites. The percentage inhibition of 20S proteasome activity in human blood cells is a reliable measure of the biologic activity of PS-341. Significant responses to PS-341 have been observed in patients with hematologic malignancies, including in those with advanced refractory multiple myeloma and AML. The antineoplastic effect of PS-341 appears to involve several mechanisms, including the inhibition of cell growth signaling pathways and cellular adhesion molecule expression, the induction or facilitation of apoptosis, and an antiangiogenic action possibly mediated by altering stromal cell-malignant cell interactions, with a consequent decrease in VEGF production.

**Targeting Angiogenesis Modulators**

If one focuses on VEGF, several obvious points of intervention emerge: decreasing production (antisense strategy or ribozymes), blocking receptor binding (anti-VEGF monoclonal antibodies), and blocking signal transduction. Indirect methods of VEGF suppression include COX-2 inhibition, UPP inhibition, and methods mediated by compounds such as aplidine (APL), in which the mechanism of action is not fully elucidated.

**Angiogenesis Inhibitors**

Bevacizumab (Avastin) is an anti-VEGF monoclonal antibody composed of a humanized murine antibody with antigen binding complementary determining regions from murine VEGF A.4.6.1. It recognizes all VEGF isoforms but does not bind to bFGF, HGF, or platelet-derived growth factor (PDGF). It has potent indirect antitumor activity in experimental models. In a phase I, dose-escalation study involving 25 patients with refractory solid tumors, bevacizumab (0.1 to 10 mg/kg) was administered as a 90-minute infusion on days 0, 28, 35, and 42. Dose-limiting toxicities were not observed at weekly doses of 10 mg/kg or less, although some patients experienced mild to moderate asthenia, mild headache, fatigue, nausea, and low-grade fever on the day of drug administration. In addition, bleeding at tumor sites developed in three (12%) patients.

No data on single-agent bevacizumab in patients with hematologic malignancies have been published; a study in MDS patients is ongoing. A National Cancer Institute-sponsored phase II study in patients with blast phase CML, in which bevacizumab is given both prior to and with idarubicin (Idamycin) and cytarabine, is ongoing. In addition, a planned study will examine the combination of bevacizumab and thalidomide in patients with refractory multiple myeloma.

A number of VEGF-receptor kinase inhibitors are in clinical development, including SU5416, SU6668, SU11248, PTK787/ZK 222584, ZD6474, and CGP41251, of which SU5416 is the furthest in clinical
development for patients with hematologic malignancies. SU5416 binds to the adenosine triphosphate binding site of VEGFR-1 and VEGFR-2.\[45,46\] In addition, it also binds to PDGF receptor and c-kit, a growth factor that promotes the survival of early hematopoietic progenitor cells and acts synergistically with other hematopoietic growth factors across multiple lineages.\[47\] The inhibition of c-kit is an emerging important therapeutic target in patients with AML.\[48\]

Another receptor tyrosine kinase of increasing interest for treating AML is the Flt-3, which is expressed on leukemic blasts and mediates survival and proliferation. Internal tandem duplications in the Flt-3-juxtamembrane region, which result in constitutive kinase activity, are found in approximately 30% of AML patients, and their presence is associated with a poor prognosis. SU5416 inhibits phosphorylation of internal tandem duplication mutant Flt-3 both in vitro and in xenograft mouse models.

SU5416 produces a dose-dependent inhibition of tumor growth in a variety of xenograft models. In a model of AML, SU5416 inhibited the stem cell factor-induced proliferation of MO7e cells.\[49\] Incubation of MO7e cells with SU5416 induces apoptosis through activation of caspase-3 and increased poly (ADP-ribose) polymerase cleavage. These data were reproducible in blasts from patients with AML. A phase I study established that the maximum tolerated dose of SU5416 was exceeded on a regimen of 190 mg/m²/d intravenously twice weekly for 4 weeks. The dose-limiting toxicities were projectile vomiting, headache, and nausea, all of which were reversible over a 24- to 48-hour period. Mild to moderate toxicities included headache, pain at the infusion site, phlebitis, change in voice, and elevated aminotransferase levels.

Ongoing studies in patients with hematologic malignancies are being conducted at a dose of 145 mg/m² twice weekly on a 4-week cycle. In a pilot study of SU5416 in 14 evaluable patients with c-kit-positive AML, major toxicities included 1 fatal gastrointestinal bleed, 1 grade 4 pancreatitis, 1 grade 4 hepatic transaminitis, and grade 2/3 bone pain in 3 patients.\[50\] Five patients demonstrated a partial response; however, 9 patients failed to respond, including 6 who had progressive disease during the study.

A US, multicenter, phase II trial has recently completed enrollment. Patients with refractory AML, MDS, CML, agnogenic myeloid metaplasia, and multiple myeloma were treated with SU5416 145 mg/m² twice weekly on a 4-week cycle. Objective responses have been reported in patients with AML and agnogenic myeloid metaplasia in preliminary analyses of the results. SU6668, another small-molecule inhibitor of angiogenesis, is not being clinically developed for patients with hematologic malignancies. Another agent, SU11248, is a third-generation, orally available, VEGF- and PDGF-receptor tyrosine kinase inhibitor due to enter phase I/II studies in 2002 in patients with refractory hematologic malignancies.

The agent PTK787/ZK222584 is also a potent and relatively selective inhibitor of VEGFR-1 and VEGFR-2 and is active in the submicromolar range.\[51\] At higher concentrations, it also inhibits other class III-receptor tyrosine kinase inhibitors, including PDGF, c-kit, and c-fms. However, PTK787 is not active against receptor tyrosine kinase from other families or intracellular kinases. It inhibits VEGF-induced endothelial cell proliferation, migration, and survival in the nanomolar range in cell-based assays. However, PTK787 does not have cytotoxic or antiproliferative effects on cells that do not express VEGFR. The VEGF- and PDGF-induced angiogenesis are inhibited in a dose-dependent manner by PTK787 in both a growth factor implant model and a tumor cell-driven angiogenesis model after once-daily oral dosing (25 to 100 mg/kg). In the same dose range, PTK787 also inhibits the growth of several human carcinomas in animal models by inhibiting microvessel formation in the interior of the tumor. However, PTK787 does not impair wound healing and has no overt effects on circulating blood cells or bone marrow leukocyte levels.

In ongoing phase I studies, patients have been treated at an oral dose range of 50 mg to 2,000 mg once daily and 150 mg twice daily on a continuous dosing schedule. In addition, phase I/II studies in patients with refractory hematologic malignancies are due to begin in 2002.

**Conclusions**
It is immediately apparent that one or more of the angiogenesis inhibitors exhibit sufficient clinical activity in patients with hematologic malignancies to stimulate large-scale commercial interest in further clinical development. The traditional bias against development of drugs in these uncommon hematologic disorders (in favor of development in solid tumors) must be resisted—in vitro evidence that angiogenesis is important in leukemia is more convincing than that for any other human malignancy. Novel clinical trial designs are clearly needed. The multiple signaling pathways activated by VEGF, including mitogen-activated protein kinase, focal adhesion kinase, phosphatidylinositol 3-kinase, protein kinase B, protein kinase C, and paxillin, are each being independently investigated as potential therapeutic targets. In addition, combinations of VEGF inhibitors with PS-341 appear particularly promising.

References:


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