

Antiangiogenesis in haematological malignancies

William W. Li,¹ Michelle Hutnik¹ and Gerald Gehr^{1,2,3}

¹The Angiogenesis Foundation, Cambridge, MA, ²Department of Medicine, Dartmouth Medical School, Lebanon, NH, and

³Department of Hematology-Oncology, Department of Veterans Affairs Medical Center, Manchester, NH, USA

Summary

Angiogenesis, the growth of new capillary blood vessels, is a central regulator of cancer growth, and a validated target for cancer therapy. The antiangiogenic agents in clinical use target one or more cellular pathways involved in the cascade of vascular growth. In haematological malignancies, angiogenesis occurs within a bone marrow ecosystem comprised of closely apposed malignant cells, endothelial cells, pericytes, fibroblasts, endothelial progenitor cells, dendritic cells, and extracellular matrix. Inhibition of angiogenesis therefore blocks not only the delivery of oxygen and micronutrients to cancer cells, but also disrupts the interdependency of these cellular players and the paracrine effects they exert to maintain the malignant phenotype. Agents such as thalidomide, lenalidomide, bortezomib, and bevacizumab, have demonstrated clinical activity in myeloma, myelodysplastic syndrome, and leukaemias. In leukaemia, vascular endothelial growth factor (VEGF) is emerging as a compelling biological target for therapy, as well as a potential predictive marker for disease relapse. Initial clinical studies suggest that the anti-VEGF strategies may advance the primary, sequential or adjunctive treatment for leukaemia, and establish the basis for other potential antiangiogenic strategies in haematological malignancies.

Keywords: angiogenesis, antiangiogenesis, haematogenous malignancies, vascular endothelial growth factor, leukaemia.

Angiogenesis, the growth of new capillary blood vessels, is a central regulator of cancer growth. The tumor vasculature delivers oxygen and micronutrients to proliferating cancer cells, and both tumor and endothelial cells release autocrine and paracrine signals that influence their microenvironment. The concept of 'antiangiogenesis' was pioneered in 1971 by Judah Folkman based on his observation that experimental solid tumors restricted from a blood supply were unable to expand (Folkman, 1971, 2007). Subsequently, the identification of specific angiogenic factors, such as vascular endothelial growth factor (VEGF), released by cancer cells enabled the

development of targeted therapies to inhibit angiogenesis in cancer patients. In 2003, bevacizumab, an anti-VEGF monoclonal antibody, became the first antiangiogenic therapy to be validated as a cancer therapy, heralding the arrival of a fourth modality for cancer treatment, after surgery, radiation, and chemotherapy. A growing number of antiangiogenic agents, including tyrosine kinase inhibitors (sunitinib; sorafenib), anti-cytokine drugs (thalidomide; lenalidomide), and a proteasome inhibitor (bortezomib) (see Table I), have now entered clinical practice, and more than 100 agents are undergoing clinical or preclinical development.

The process of angiogenesis in haematological malignancies shares similarities with neovascularization of solid tumors. Endothelial cells within pre-existing venules are activated, proliferate, migrate, and form vascular tubes. While aberrant compared to normal physiological blood vessels, malignant angiogenesis in bone marrow recapitulates developmental vessel formation with arterial-venous differentiation, vascular maturation, and the recruitment of endothelial progenitor cells that reside within bone marrow vascular niches. Complex and as yet poorly characterized interactions occur between the vasculature and normal and malignant cells, the extracellular matrix, growth factors and cytokines, in the bone marrow. These interactions underlie the basis for antiangiogenic therapy in haematological disease.

This review will discuss antiangiogenic therapy in the context of haematological malignancies, with a focus on leukaemia. The activity of the three antiangiogenic agents (thalidomide, lenalidomide, and bortezomib) in their approved indications (multiple myeloma and myelodysplastic syndromes) will be presented as proof of principle of the efficacy of antiangiogenic therapies in haematological malignancies. Their activity supports the mounting body of evidence of the key role that VEGF plays in the pathology of bone marrow angiogenesis in leukaemia.

Targeting angiogenesis in bone marrow

Increased bone marrow vascularity reflects angiogenesis induced by multiple myeloma, myelodysplastic syndromes (MDS), leukaemia, and other haematological cancers (Vacca *et al*, 1994; Perez-Atayde *et al*, 1997; Pruneri *et al*, 1999; Padro *et al*, 2000; Sezer *et al*, 2000; Keith *et al*, 2007). A large body of

Correspondence: William W. Li, The Angiogenesis Foundation, 124 Mount Auburn Street, Suites 200N, Cambridge, MA 02138, USA.

E-mail: wli@angio.org

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Table 1. FDA-approved antiangiogenic therapies.

Generic name (brand name: company)	Indication	Date approved (country)	Dosing schedule
Cancer treatments			
Bevacizumab (Avastin: Genentech)	Metastatic colorectal cancer	2/2004 (USA)	5 mg/kg i.v. every 2 weeks + 5-FU and Leucovorin or FOLFOX or FOLFIRI 7.5 mg/kg i.v. every 3 weeks + CapeOX 15 mg/kg i.v. every 3 weeks + paclitaxel and carboplatin
Bortezomib (Velcade: Millennium)	Metastatic non-small cell lung cancer – first line	10/2006 (USA)	
	Metastatic breast cancer Multiple myeloma – second line	3/2007 (EU) 5/2003 (USA)	10 mg/kg i.v. every 2 weeks + paclitaxel 3- to 5-second bolus 1.3 mg/m ² i.v. twice weekly for 2 weeks followed by a 10-d rest period (21 d cycle)
Cetuximab (Erbix: ImClone/BMS)	Mantle cell lymphoma – second line	12/2006 (USA)	1.3 mg/m ² i.v. 3- to 5-second bolus twice weekly for 2 weeks followed by a 10-d rest period (21 d cycle)
	Metastatic colorectal cancer – second line	2/2004 (USA)	400 mg/m ² initial loading dose 250 mg/m ² weekly
Endostar (rh-endostatin: Simcere Pharmaceuticals)	Locally advanced or metastatic head and neck cancer	12/2005 (Switzerland)	400 mg/m ² initial loading dose 250 mg/m ² weekly with or without radiation
	non-small cell lung cancer – first and second line	9/2005 (China)	7.5 mg/m ² days 1 to 14 in 3 week cycle + vinorelbine + cisplatin
Erlotinib (Tarceva: Genentech/OSI/Roche)	Locally advanced or metastatic non-small cell lung cancer – second line	11/2004 (USA)	150 mg/d
	Pancreatic cancer – first line Multiple myeloma – second line	11/2005 (USA) 6/2006 (USA)	100 mg/d + gemcitabine 25 mg/d capsule on Days 1 to 21 of repeated 28-d cycles + dexamethasone
Sorafenib (Nexavar: Bayer/Onyx)	Deletion 5q myelodysplastic syndrome	12/2005 (USA)	10 mg/d capsule on Days 1 to 21 of repeated 28-d cycles
	Advanced renal cell carcinoma – second line Unresectable hepatocellular carcinoma	12/2005 (USA) 10/2007 (EU)	400 mg twice per day 400 mg twice per day
Sunitinib Malate (Sutent: Pfizer)	Advanced renal cell carcinoma – first line Gastrointestinal stromal tumor	1/2006 (USA) 1/2006 (USA)	50 mg once daily for 4 weeks, followed by 2 weeks off treatment 50 mg once daily for 4 weeks, followed by 2 weeks off treatment
	Erythema nodosum leprosum Multiple myeloma	6/1998 (USA) 12/2003 (Australia)	100–400 mg/d 200 mg/d + dexamethasone

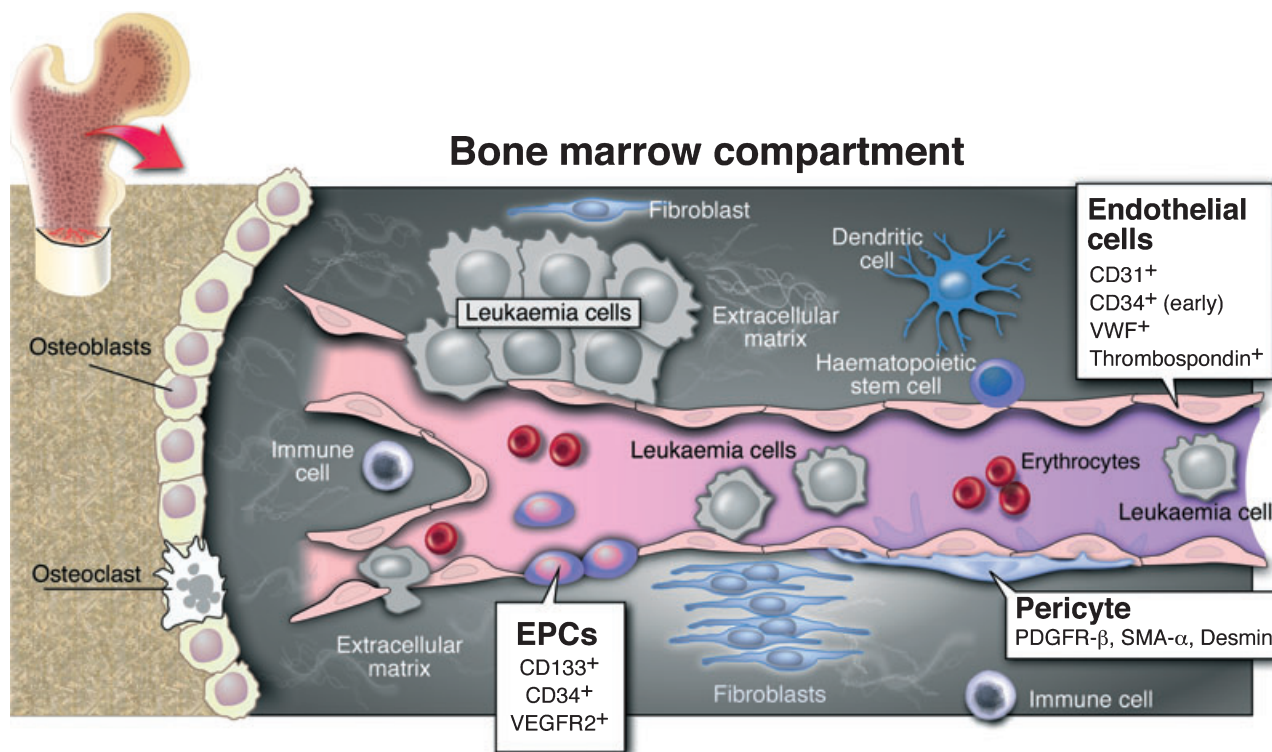


Fig 1. Antiangiogenic therapies in haematological cancers can target multiple cells and pathways in the bone marrow microenvironment, comprised of extracellular matrix (fibronectin, laminin, collagen) tightly interdispersed with vascular endothelial cells, pericytes, hematopoietic stem cells, endothelial progenitor cells (EPCs), immune cells (including Natural Killer cells, T lymphocytes, and monocytes), stromal cells (fibroblasts), dendritic cells, osteoblasts, and osteoclasts. Antiangiogenic agents interfering with one cell or pathway can disrupt other interdependent cells and pathways.

evidence links the extent of neovascularization observed in bone marrow biopsy specimens with disease burden, prognosis, or treatment outcome (Rajkumar *et al*, 2002).

Physiological angiogenesis in the bone marrow is induced and maintained by the balanced interplay of cells, cytokines and growth factors within a complex ecosystem in which endothelial cells and pericytes proliferate in concert with fibroblasts, dendritic cells, inflammatory cells, and haematopoietic stem cells. Malignant cells in the bone marrow upset this balance by producing and responding to the same factors, thereby stimulating angiogenesis, leading to increased vascularity. (see Fig 1). Several factors, including interleukin-6, granulocyte-macrophage colony-stimulating factor and VEGF, have autocrine and paracrine effects acting on multiple cell types. The proliferation of bone marrow capillaries contributes to the interdependency of these cell populations, and their interruption by antiangiogenic agents interferes with cell-cell, cell-matrix, and cell-cytokine/growth factor interactions in a fashion distinct from that observed in solid tumors.

A growing number of agents that inhibit angiogenesis are demonstrating clinical activity in haematological cancers. Thalidomide, an immunomodulatory antagonist of tumour necrosis factor- α , inhibits endothelial cell activation by VEGF, basic fibroblast growth factor (bFGF), and other pro-angiogenic factors (D'Amato *et al*, 1994). Lenalidomide is a potent thalidomide analog that, like thalidomide, has a

complex mechanism of action that includes antiangiogenic activity.

Bortezomib, a proteasome inhibitor and antagonist of the antiapoptotic nuclear factor κ -B pathway, induces endothelial cell apoptosis and represses hypoxia-inducible factor 1 and VEGF production (Williams *et al*, 2003; Shin *et al*, 2008). Bevacizumab is a monoclonal antibody that neutralizes VEGF and is highly active in numerous cancers (Ferrara *et al*, 2004).

The clinical studies of haematological cancers in which these agents have demonstrated activity will be reviewed.

Multiple myeloma

Thalidomide was the first angiogenesis inhibitor to demonstrate clinical efficacy in multiple myeloma (D'Amato *et al*, 1994; Anargyrou *et al*, 2008). Specifically in myeloma, thalidomide down-regulates VEGF secretion from bone marrow endothelial cells obtained from patients with active disease. In a landmark Phase 2 clinical trial, 169 previously treated patients with refractory myeloma received thalidomide monotherapy (Singhal *et al*, 1999; Barlogie *et al*, 2001). Partial response, defined as 50% paraprotein reduction, was achieved in 30% of patients, and 14% achieved a complete or nearly complete remission. The survival rate at 2 years was 48%. These results led to many subsequent clinical studies of thalidomide in myeloma, leading ultimately to Federal Drug

Administration (FDA) approval of the drug in 2006, for the treatment of newly diagnosed multiple myeloma, in combination with dexamethasone. In the pivotal Phase 3 trial, the response rate in patients receiving thalidomide plus dexamethasone was 63% compared to 41% with dexamethasone alone ($P = 0.0017$) (Rajkumar *et al*, 2006). Long-term outcome measures, including time-to-progression (TTP) and progression-free survival (PFS), were recently reported for a 470 patient randomized, placebo-controlled Phase 3 clinical trial of a similar protocol in newly diagnosed multiple myeloma, with comparable overall response rates (Rajkumar *et al*, 2008). Significant increases resulted in both median TTP (22.6 vs. 6.5 months; $P < 0.001$) and median PFS (14.9 vs. 6.5 months; $P < 0.001$) for the thalidomide plus dexamethasone group *versus* dexamethasone alone.

Lenalidomide, in combination with dexamethasone, is FDA approved for the treatment of multiple myeloma in patients who have received at least one prior therapy. This agent inhibits angiogenesis by antagonizing the production of VEGF in the bone marrow milieu (Richardson *et al*, 2002). A retrospective analysis of pooled data from two Phase 3 clinical trials (MM009 and MM010) enrolling a combined total of 692 patients with previously treated relapsed/refractory multiple myeloma demonstrated an improved response rate (59.2% vs. 22.5%; $P < 0.001$) and increased median TTP (48.1 vs. 20.1 weeks; $P < 0.001$) for patients treated with lenalidomide and dexamethasone, compared with dexamethasone alone (Wang *et al*, 2006; Dimopoulos *et al*, 2007; Weber *et al*, 2007). The toxicities of lenalidomide are less severe and less frequent than those of its analog, thalidomide.

Bortezomib is also FDA-approved for the treatment of myeloma that has relapsed after two prior treatments, or where resistance has developed following the last treatment. A first-in-class proteasome inhibitor, bortezomib inhibits VEGF secretion in endothelial cells derived from patients with myeloma (Roccaro *et al*, 2006). In a Phase 3 trial involving 669 myeloma patients treated with at least one prior therapy, bortezomib increased median TTP (6.2 months vs. 3.5 months; $P < 0.0001$), improved overall survival, and increased response rate (38% vs. 18%; $P < 0.0001$), compared with high-dose dexamethasone (Kane *et al*, 2006).

Myelodysplastic syndromes

In myelodysplastic syndromes, bone marrow dysfunction causes underproduction of erythrocytes, leucocytes, and platelets, leading to anemia, infection, or bleeding. The three-year survival rate of patients with severe MDS is as low as 35%, with mortality often caused by infection or hemorrhage (Ma *et al*, 2007). Transformation from MDS to acute myeloid leukaemia (AML) occurs in up to 40% of patients, and carries a poor prognosis. VEGF is overexpressed by immature myeloid cells in the bone marrow of patients with MDS, and this is associated with increased bone marrow vascularity and the presence of neoplastic cells (Bellamy *et al*, 2001).

An early clinical study of thalidomide in MDS resulted in a 56% hematological response, with some patients achieving transfusion independence (Strupp *et al*, 2002). Lenalidomide is FDA-approved for the treatment of transfusion-dependent anemia due to Low- or Intermediate-1-risk MDS associated with a deletion 5q cytogenetic abnormality (del 5q MDS) with or without additional cytogenetic abnormalities. In MDS, lenalidomide directly targets the malignant clone as well as the bone marrow microenvironment. Specifically for del 5q MDS, lenalidomide inhibits the growth of del 5q progenitor cells and upregulates SPARC (secreted protein acidic and rich in cysteine), which is an antiangiogenic, antiproliferative and anti-adhesive protein that regulates extracellular matrix interactions (Pellagatti *et al*, 2007). In a study of 43 MDS patients with transfusion-dependent or symptomatic anemia, 56% of the patients treated with lenalidomide exhibited a therapeutic response (List *et al*, 2005). For patients with del 5q MDS, there was an 83% response rate, with 75% of those achieving a complete cytogenetic remission. In a larger study of 148 patients with del 5q MDS, lenalidomide treatment resulted in a 76% response rate, with 45% of 85 evaluable patients achieving complete cytogenetic remission (List *et al*, 2006).

Leukaemia

The evidence that angiogenesis plays a pathophysiological role in leukemia has been well documented [for reviews, see (Bellamy *et al*, 1999; Fiedler *et al*, 1997; Folkman *et al*, 2001; Giles, 2002; Hussong *et al*, 2000; Kessler *et al*, 2007; List, 2001)] Angiogenesis in leukaemia is documented by increased bone marrow microvessel density (MVD), and increased expression of hypoxia-inducible factor 1 α (HIF-1 α), multiple angiogenic factors (VEGF, bFGF, angiopoietin-2), soluble vascular endothelial growth factor receptors (Flt-1, KDR), and decreased expression of endogenous angiogenesis inhibitors, such as thrombospondin-1 (Dong *et al*, 2007; Frater *et al*, 2008). A clinicopathological study of 40 children with newly diagnosed, untreated acute lymphoblastic leukaemia (ALL) documented a sixfold increase in leukaemic bone marrow MVD compared to bone marrow from control children ($P \leq 0.0001$) (Perez-Atayde *et al*, 1997). Human leukaemia cells (B-cell, T-cell ALL) injected into non-obese diabetic severe combined immunodeficiency (NOD/SCID) mice induced bone marrow neovascularization (Veiga *et al*, 2006). Likewise, bone marrow endothelial cells exposed to plasma derived from bone marrow of ALL patients underwent proliferation, migration, and tube formation, in contrast to lack of activity following exposure to bone marrow plasma from normal patients. ALL cells co-cultured with bone marrow endothelial cells significantly reduced leukaemia cell apoptosis, whereas in control conditions, cultured ALL cells underwent spontaneous cell death. Therefore, leukaemia cells induce the angiogenic phenotype, while endothelial cells promote leukaemia cell survival and expansion. Furthermore, leukaemic bone marrow vasculature exhibits abnormal features similar to those

characteristics of solid tumors. Myeloid leukaemia cells implanted into a cranial window of SCID mice induced neovascularization in which microvessels were tortuous and hyperpermeable, hallmarks of tumor angiogenesis (Schaefer *et al*, 2008).

The biological relationship between leukaemia and blood vessels is further evidenced by the homing of leukaemic cells to bone marrow vascular niches, where they take residence. In experimental mice injected with ALL cells, this homing is mediated by stromal-cell-derived factor 1 (SDF-1) and its receptor CXCR4 (Sipkins *et al*, 2005). CXCR4 is highly expressed on the surface of ALL cells, and on endothelial progenitor cells. The SDF-1 ligand is highly expressed by endothelial cells found in the bone marrow vascular "hot spots" or niches attracting ALL cells. *In vitro*, ALL cells also home towards bone marrow endothelial cells assembled in Matrigel, a classic assay for angiogenesis (Veiga *et al*, 2006).

VEGF as an angiogenesis target in leukaemia

As in other cancers, VEGF is a target for antiangiogenic agents in leukaemia. In addition to its well-defined effects on vascular endothelial cells, VEGF has direct and indirect effects on haematopoietic stem cells (HSC), endothelial progenitor cells, immune cells (including Natural Killer cells, T lymphocytes, and monocytes), dendritic cells, stromal cells, and in the case of neoplasia, malignant cells (Podar & Anderson, 2005). The VEGF pathway is involved in paracrine, autocrine, and juxtacrine signaling of multiple growth factors, and in regulating haematopoiesis by mediating HSC survival and differentiation (Gerber & Ferrara, 2003). VEGF inhibits dendritic cell maturation, modulates immune responses, influences bone homeostasis, and mediates progenitor cell recruitment and differentiation. VEGF is also an autocrine regulator of some cancer cells, (Dias *et al*, 2002; Vales *et al*, 2007) and is expressed in various leukaemia cell lines, including ALL, AML, acute T-cell leukaemia, chronic myeloid leukaemia (CML), B-cell chronic lymphocytic leukaemia (B-CLL), promyelocytic leukaemia, plasma cell leukaemia, as well as in leukemia blasts. Both internal and external autocrine VEGF loops have been found to mediate leukemia cell survival and proliferation (Santos & Dias, 2004). Therefore, VEGF directed therapy in leukaemia has multiple targets in the bone marrow milieu.

Circulating VEGF levels have been shown to have prognostic value in studies of pediatric and adult leukemia (Aguayo *et al*, 2002; Faderl *et al*, 2005; Avramis *et al*, 2006). Caution needs to be taken in viewing studies employing serum VEGF levels, as serum levels may overestimate true circulating VEGF levels because of the release of sequestered VEGF from platelets (Banks *et al*, 1998). However, comparison studies using serum levels involving control groups may still yield trends and valuable insights. In a study of newly diagnosed cases of pediatric ALL ($n = 31$), children with higher cellular VEGF levels at diagnosis had a poorer prognosis, with elevated VEGF

levels correlating to a 10-fold decrease in the relapse-free interval (>10 years vs. 1.2 years) (Koomagi *et al*, 2001). Five of the study patients who relapsed had VEGF levels that were higher at relapse than upon initial diagnosis. In a clinical study (CCG-1962) of native *versus* pegylated-asparaginase therapy in standard-risk ALL pediatric patients ($n = 117$), high or increasing levels of VEGF serum during induction correlated with poorer survival and more frequent events (central nervous system or bone marrow relapse) (Avramis *et al*, 2006). By contrast, ALL children with low VEGF levels during induction experienced longer event-free survival. At the end of induction, high VEGF levels (>60 pg/l) correlated with worse survival compared to low VEGF levels (<60 pg/l) ($P < 0.0001$). VEGF secreted by AML cells obtained at presentation was an independent prognostic factor for duration of relapse free survival in 47 children with newly diagnosed AML, following treatment using intensive chemotherapeutic protocols from the Dutch Childhood Leukaemia Study Group (de Bont *et al*, 2002). High VEGF secretion correlated with shorter relapse free survival, irrespective of cytogenetic abnormalities, white blood cell count, French-American-British (FAB) classification, risk assessment, and age at diagnosis.

Similar associations have been documented in adult leukaemia. In 58 adults with previously untreated AML, increased levels of plasma VEGF correlated with reduced survival ($P = 0.02$) and lower complete remission rates ($P = 0.004$) (Aguayo *et al*, 2002). Pretreatment cellular VEGF concentrations obtained from peripheral blood and bone marrow samples from newly diagnosed adult AML patients ($n = 99$) with high leucocyte and blast counts were prognostic in multivariate analysis (Aguayo *et al*, 1999). In another larger study of adult AML ($n = 133$), high plasma levels of soluble VEGF receptor-1 (sVEGFR1 or sFlt-1) was associated with more aggressive disease, while lower levels correlated with complete responses to treatment (Hu *et al*, 2004). Assessment of pretreatment bone marrow cellular levels of VEGF in chronic phase CML patients ($n = 148$) revealed an inverse relationship between VEGF levels and survival (Verstovsek *et al*, 2002). Taken together, these multiple lines of scientific, laboratory, and clinicopathological evidence support the rationale for VEGF-directed antiangiogenic therapy in leukaemia.

Clinical experience with antiangiogenic therapy in leukaemia

Several types of approved angiogenesis inhibitors have been tested in adult leukaemia patients: anti-cytokine agents, proteasome inhibitors, multikinase inhibitors, a monoclonal antibody, and mTOR (mammalian target of rapamycin) inhibitors. Each of these targets has been implicated in leukemia and are downstream (or upstream) in the VEGF signaling cascade.

Thalidomide has been clinically evaluated in advanced AML patients, both as monotherapy and combination with

chemotherapy (Steins *et al*, 2003; Barr *et al*, 2007). Although single-agent thalidomide has both antiangiogenic and anti-leukaemic activity in AML, its clinical efficacy is modest, with response rates of only between 6% and 24%. The limited responses coupled with toxicities associated with thalidomide, such as sedation, neuropathy, and constipation, have limited its development for leukaemia. The less toxic lenalidomide has activity as monotherapy in relapsed/refractory CLL patients. A small study ($n = 44$) showed an overall response rate of 32%, with 7% patients who achieved a complete response, 23% a partial response, and 2% a nodular partial remission (Ferrajoli *et al*, 2008). Bortezomib, administered to AML patients ($n = 31$) in combination with idarubicin and cytarabine led to complete responses in 61% of patients, with a good safety profile (Attar *et al*, 2008). Serious pulmonary and cardiac toxicities have been associated with bortezomib therapy, and significant precautions for its use in patients with pulmonary or pericardial disease are recommended.

Small molecule tyrosine kinase inhibitors are an important class of antiangiogenic agents. SU5416 inhibits the kinase signaling of VEGFR-1,-2, c-kit, and Flt3. This agent was studied in 43 patients with advanced AML demonstrating an overall response rate of 19%, with one complete morphological response lasting 2 months (Fiedler *et al*, 2003). Another multikinase angiogenesis inhibitor, PTK787, targets VEGFR-1,-2,-3, platelet-derived growth factor receptor (PDGFR), and c-kit signaling. PTK787 was studied in a Phase 1 clinical trial alone or in combination with cytosine arabinoside and daunorubicin (upfront or if monotherapy was ineffective) in patients with advanced MDS or AML. PTK787 monotherapy was administered (Arm 1; $n = 18$) in patients with primary refractory or relapsed AML. In a second arm of 45 patients with secondary AML, poor-prognosis de novo AML or advanced MDS, patients received PTK787 monotherapy (Arm 2M; $n = 35$), or combination therapy (Arm 2C; $n = 17$) upfront or following monotherapy. Although there were no responses in Arm 1, in Arm 2M there were two patients who achieved SD for 10–14 months. In Arm 2C, there were five complete remissions, two complete remissions (platelets not recovered), and one partial remission (Roboz *et al*, 2006).

Sunitinib is a multikinase inhibitor of VEGFR-1,-2, PDGFR, Flt3, and c-kit that is approved for renal cell carcinoma and gastrointestinal stromal tumors. It was evaluated in a Phase 1 clinical trial in refractory AML patients ($n = 15$) (Fiedler *et al*, 2005). In four patients with Flt3 mutations, all had morphologic or partial responses of short duration. Flt3 activation stimulates haematopoiesis, leukaemia cell proliferation, and endothelial cell proliferation (Drexler, 1996). Flt3 inhibition in leukaemia cell lines suppressed VEGF production in a dose-dependent fashion (Lopes de Menezes *et al*, 2005). Approximately one-third of AML patients have activating Flt3 mutations, which are associated with poor outcome. Some patients with non-mutated Flt3 also show evidence of Flt3 activation (Knapper, 2007). Several Flt3 inhibitors have been

studied in AML clinical trials, exhibiting modest single agent activity. Combinatorial therapy trials, combining Flt3 inhibitors and cytotoxic agents, in AML are underway. Sorafenib, another multikinase agent, is in clinical trials for AML, ALL, CML, and MDS.

Bevacizumab, a partially-humanized IgG monoclonal antibody that neutralizes VEGF-A, is an approved antiangiogenic therapy for cancers of the colon, lung, and breast. Bevacizumab administered as monotherapy in patients with heavily-treated refractory AML ($n = 9$) resulted in a time-dependent reduction in VEGF expression observed by bone marrow immunohistochemistry, but without a clinical response (Zahiragic *et al*, 2007). This is consistent with the vast majority of clinical studies in refractory solid tumors in which single agent bevacizumab is inadequate to achieve improvement in clinical outcome (Quesada *et al*, 2007).

When bevacizumab was combined, however, into timed sequential therapy involving 1- β -D-arabinofuronosylcytosine (ara-C) and mitoxantrone, an improved overall response rate of 48% resulted, including complete responses (33%) and partial responses (14.5%) (Karp *et al*, 2004). In this Phase 2 study, ara-C was administered on day 1, followed by mitoxantrone (day 4), then bevacizumab (day 8). Serum VEGF levels decreased within 2 h following bevacizumab administration, and bone marrow MVD declined over the course of 7 d. Ara-C itself may be converted into an angiogenesis inhibitor by replacing the oxygen atom in the arabinose sugar ring with a sulphur atom to create the structural analog, T-ara-C (Roy *et al*, 2006).

Inhibitors of mTOR are another important class of antiangiogenic agents. These include: deforolimus, everolimus, rapamycin (sirolimus), and temsirolimus (Giles & Albitar, 2005; Martelli *et al*, 2007). Rapamycin and related mTOR inhibitors inhibit endothelial cell VEGF expression, as well as VEGF-induced endothelial cell proliferation (Dormond *et al*, 2007). In a pilot study of six patients with imatinib-resistant CML, rapamycin induced major and minor leucocyte responses, with an observed decrease in *VEGFA* mRNA levels in circulating leukaemic cells (Sillaber *et al*, 2008). Another study of rapamycin in adult refractory/relapsed AML showed partial responses (>50% reduction in the absolute number of blood blasts or $\geq 0\%$ reduction in the percentage of marrow blasts) and stable disease (Recher *et al*, 2005). Deforolimus has been studied in a Phase 2 trial in pretreated patients with various hematological malignancies, including ALL, AML, CLL, CML, MDS, agnogenic myeloid metaplasia, mantle cell lymphoma and T-cell leukaemia/lymphoma (Rizzieri *et al*, 2008). Overall, 40% of deforolimus-treated patients experienced hematological improvement or stable disease.

Other antiangiogenic agents under testing in adult leukaemias include AZD2171, enzastaurin, homoharringtonine, and UCN-01. Homoharringtonine, a myelosuppressive plant alkaloid isolated from the Plum yew tree *Cephalotaxus*, downregulates VEGF in leukaemic cells, and has demonstrated clinical activity, including induction of complete remissions, in

patients with relapsed CML (Quintas-Cardama *et al*, 2007). The activity of these agents, and their value in comparison to existing therapies, remains to be established in future well-designed clinical trials.

Future directions

Because endothelial cell targets exist across all cancer types, antiangiogenic agents that are validated as safe and effective in solid tumors are logical candidates for testing in haematological malignancies. In addition to bevacizumab, sunitinib, and sorafenib, these approved agents include cetuximab, everolimus, recombinant human endostatin, erlotinib, and temsirolimus. Clinicians treating haematological malignancies should give due consideration to the entire class of antiangiogenic agents as having potential utility in disease management.

Identifying new targets and understanding their mechanisms unique to pathological bone marrow angiogenesis will pave new approaches to antiangiogenic therapy (Nimer, 2008). Novel endothelial targets might include growth factors and their receptors, adhesion molecules, and endothelial-secreted cytokines. Targeted disruption of paracrine and autocrine loops of cytokine, and adhesion-mediated signaling pathways may further amplify the anti-cancer effect in leukaemia, myeloma, and MDS. Studies of the genetic and epigenetic makeup of patients who are best- or non-responders to specific antiangiogenic therapies will reveal additional clinical features important for optimizing this modality.

Although oncology agents are primarily aimed at adult cancers, paediatric haematological malignancies are particularly important opportunities for antiangiogenic therapy development. Anti-VEGF agents, in combination with antimetabolite or cytotoxic drugs, clearly have activity in paediatric leukemia. The overall safety profile of angiogenesis inhibitor drugs represents an important feature for application to children, especially if relapse or even secondary cancers can be prevented with long-term, adjuvant or maintenance treatment. Because bone marrow is a reservoir for haematopoietic stem and other progenitor cells, treatment specificity to disease pathways will be important for minimizing side effects, including normal growth and development. Ultimately, it should be possible to exploit the principles of antiangiogenic therapy to generate interventions that can be used to induce remissions and subsequently, to prevent relapse through long-term maintenance therapy. The successful development of this application of antiangiogenesis would validate Folkman's original hypothesis of maintaining cancer in a permanent state of dormancy, in the setting of haematological malignancies.

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