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Review Article

Current status of anti-vascular endothelial growth factor (VEGF) strategies and future directions

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CONTENTS

Abstract

Angiogenesis, or the process by which new vessels are created from preexisting vasculature, has become the subject of intense research in recent years. Increased rates of angiogenesis are associated with several disease states, including cancer, age-related macular degeneration (AMD), psoriasis, rheumatoid arthritis and diabetic retinopathy. Numerous growth factors, including vascular endothelial growth factor (VEGF), are able to induce angiogenesis. Overexpression of VEGF has been correlated with increased metastasis of tumors and is a marker of poor prognosis in many carcinomas. Various therapies have been designed to target VEGF and inhibit angiogenesis, including monoclonal antibodies, soluble receptor decoys, tyrosine kinase receptor (TKR) inhibitors and other therapies targeting the mRNA that codes VEGF. Clinical trials have shown VEGF inhibitors to be effective in increasing overall survival rates, time to tumor progression, partial response rates and duration of response in cancer patients. In AMD patients, these therapies slow the rate of vision loss and in some cases increase visual acuity. Although these therapies are a milestone in the treatment of these disease states, several concerns need to be addressed before their impact can be fully understood.

Introduction

Angiogenesis is a term used to describe the formation of new blood vessels from the preexisting vasculature. This process is critical for several normal physiological functions, including the development of embryos, wound healing, the female reproductive cycle and collateral vascular generation in the myocardium. However, aberrant angiogenesis has been implicated in the progression of several disease states, including cancer, macular degeneration, diabetic retinopathy, rheumatoid arthritis and psoriasis.

Under normal physiological conditions, the process of angiogenesis is well controlled and a perfect balance between endogenous angiogenic growth factors and suppressors exists. When angiogenic growth factors outnumber angiogenesis inhibitors, the balance shifts in favor of angiogenesis and this has been termed the "angiogenic switch" (1).

Rigorous research in the field of angiogenesis has led to the identification of many regulators involved in angiogenesis. Angiogenesis is driven by the production of proangiogenic growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-8 (IL-8), placenta growth factor (PlGF), transforming growth factor β (TGF-β), nitric oxide synthase (NOS), angiopoietin, platelet-derived endothelial growth factor (PDEGF), pleiotrophin and several others (2). In addition, angiogenesis can be caused by a deficiency in endogenous angiogenesis inhibitors, including angiostatin, canstatin, endostatin, various heparinases, interferon alfa, beta and gamma (IFN-α, -β, -γ), thrombospondin and others (3).

Although angiogenesis is not understood in its entirety, the roles of many of its regulators and the fundamental steps that result in angiogenesis have been well documented. Initially, vascular endothelial cells (ECs) are activated by proangiogenic growth factors, which cause ECs to release proteases that degrade the basement membrane. This allows ECs to escape from the original vessel walls, proliferate and extend toward the source of the angiogenic stimulus, using integrins to cause cell

adhesion. Finally, tubule formation occurs, allowing blood to flow within the new vessel (1, 3).

Pathological angiogenesis

Cancer research has shown that due to a lack of oxygen and other essential nutrients tumor growth is limited to 1-2 mm (3, 4). In order to grow beyond this size tumor cells must induce angiogenesis by secreting various angiogenic growth factors. Angiogenic vascularization not only allows tumor growth, but also increases the rate of metastasis. Vessels formed by uncontrolled and unregulated angiogenesis are drastically different from those of the normal vasculature and are characterized by chaotic branching, hypoxia and increased interstitial pressure. These irregularities may hinder the ability of chemotherapeutic agents to achieve the desired concentration within tumor vasculature.

Age-related macular degeneration (AMD) is a progressive disease affecting the central portion of the retina (the macula), often resulting in vision loss. In the earliest stage, deposits called drusen form in the area between the retinal pigment epithelium (RPE) and the underlying choroid. Advanced AMD, which is responsible for profound vision loss, has two forms: dry and wet. The dry form of advanced AMD results from atrophy of the RPE layer below the retina, and there is currently no treatment option for this type. In wet AMD (neovascular AMD), neovascularization of the choroid occurs, resulting in blood and protein leakage. The seepage and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and can lead to vision loss (5). Angiogenic growth factors have been shown to be elevated in patients with the wet form of AMD and play a key role in the neovascularization process (6).

Research also shows that angiogenesis accompanies the progression of chronic inflammation. It has been demonstrated that VEGF is overexpressed in a number of proinflammatory conditions, including psoriasis and rheumatoid arthritis (7, 8). Thus, VEGF is an attractive target that researchers are investigating for the treatment of these diseases.

Antiangiogenic therapies

A wide range of therapies designed to inhibit angiogenesis have been developed and many more are in development. Angiogenesis inhibitors have typically been divided into two categories: either direct or indirect. Direct angiogenesis inhibitors are those therapies designed to target ECs and prevent their proliferation, and indirect therapies target the proangiogenic growth factors or their receptors.

ECs are thought be an excellent target for therapy because they are considered genetically more stable than cancer cells. It is postulated that this stability reduces the likelihood of rapid mutation and acquired drug resistance (9). Recent studies suggest, however, that genetic anomalies are present in tumor ECs and may be able to confer drug resistance (10). Interestingly, it has also been suggested that traditional therapies, such as radiation therapy, may actually work in part by targeting the genomically stable ECs, as these ECs are still proliferating at a higher than normal rate (11).

Indirect inhibition of angiogenesis can be further divided into two categories: either amplifying the effects of angiogenic inhibitors and the activation of their pathways or inhibiting the activation of proangiogenic pathways. Currently, these therapies have employed a multitude of targets, including many angiogenic regulators and their receptors. One example is a therapy designed to target TGF. A recent phase II trial investigated the use of the TGF-β antisense vaccine belagenpumatucel-L (Lucanix™) in patients with non-small cell lung cancer (NSCLC) and results appeared favorable (12). Focusing on the 61 assessable late-stage (IIIB and IV) patients, a 15% partial response rate was achieved and the estimated probabilities of surviving 1 and 2 years were 68% and 52%, respectively. These results are favorable compared to historical controls. No significant adverse events were observed.

Another therapy being explored targets TGF and employs the use of a soluble TGF-β receptor (sTGF-βR) that specifically inhibits TGF-β1 and TGF-β3 (13, 14). It is currently in the preclinical stage and looks promising. Other therapies with different targets are being developed as well.

VEGF

VEGF is a member of a family of dimeric glycoproteins that belong to the platelet-derived growth factor (PDGF) family of growth factors. While VEGF, also known as VEGF-A, is the most comprehensively studied member of the family, others include VEGF-B, VEGF-C, VEGF-D and PlGF (15, 16). VEGF-A has several isoforms ($VEGF_{121}$, $VEGF_{121b}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$) resulting from alternative splicing, of which $VEGF_{145}$ is the most abundant (17). All VEGF ligands bind to tyrosine kinase receptors (TKRs), causing the receptors to dimerize and phosphorylate (18). Upon binding to its receptor, VEGF initiates a cascade of signaling events that begins with autophosphorylation of both receptor kinases, followed by activation of numerous downstream proteins, including phospholipase C γ (PLC-γ), phosphatidylinositol 3-kinase (PI3K), GTPase-activated protein (GAP), Ras, mitogen-activated protein kinase (MAPK) and others (19). VEGF-A binds to VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) (18). VEGFR-2 has higher affinity for VEGF and one of its biological activities is the potentiation of angiogenesis. The function of VEGFR-1 is less well defined, but seems to include recruitment of monocytes and hematopoiesis (19). VEGF-C and VEGF-D bind to a different receptor, VEGFR-3, which mediates lymphangiogenesis (16).

The biological activities of VEGF have also been well documented. VEGF was discovered because of its ability to promote vascular permeability (20), and it has also

been shown to promote the growth, migration and proliferation of ECs (20-22). In addition, it induces vasodilatation and enhances EC survival (20, 21). These biological activities occur in few physiological processes other than wound healing and ovulation, making VEGF an attractive target for therapy.

Role in AMD

VEGF has been shown to be overexpressed in patients diagnosed with AMD. A study was designed to determine the effect of VEGF overexpression in RPE cells (6). A recombinant adenovirus vector expressing rat $VEGF_{164}$ was constructed and injected into the subretinal space. RPE cells increased their expression of VEGF mRNA and blood vessels became leaky 10 days postinjection. By 80 days postinjection, new blood vessels had originated from the choriocapillaris, which ultimately led to the formation of choroidal neovascular membranes and the death of photoreceptor cells. This study demonstrated that overexpression of VEGF in RPE cells can induce vascular leakage, new choroidal blood vessel growth, the development of choroidal neovascularization and neural retina degeneration (6). This is the same process by which AMD has been shown to cause vision loss, suggesting that VEGF overexpression plays a key role in AMD.

Role in cancer

VEGF has been directly correlated with increased tumor size and metastasis and shorter overall survival. As a result, VEGF overexpression has been deemed a poor prognostic indicator in many types of tumors, including those of the breast, kidney, colon, brain, ovary, cervix, bladder, esophagus, prostate and soft tissue (22-33). Consequently, therapies targeting VEGF represent a large portion of the antiangiogenic drugs being developed.

VEGF is the most thoroughly investigated proangiogenic growth factor and a sequential pathway leading to angiogenesis has been well documented. VEGF is secreted primarily in response to hypoxia or ischemia (34, 35). In these types of low-oxygen environments, hypoxia-inducible factor 1 (HIF-1) becomes stabilized and VEGF expression is upregulated due to HIF-1 binding and activation of the VEGF promoter, ultimately resulting in increased mRNA transcription (22, 35). VEGF upregulation causes activation of ECs and local vessels to become permeable. Permeability of blood vessels causes tissue edema and deposition of various cytokines into the extravascular space. Next, thin-walled vessels expressing VEGF receptors form via the proteolytic degradation of the basement membrane of existing vessels. Finally, spreading and thinning (increasing the surface area) of the preexisting vascular endothelium over the framework for the new vessel that was generated from basement membrane degradation occur (36). Although many of the steps in this pathway have been revealed, angiogenesis appears to be a complex biological process that can occur by way of several pathways. Recent reports demonstrate that redundancies exist which allow angiogenic growth factors to overcome inhibition of a single pathway (11).

VEGF inhibition

Currently, there are several approved therapeutic agents and many more are being studied which employ unique strategies to inhibit the VEGF pathway. One approach involves the use of monoclonal antibodies (mAbs) to target either VEGF itself or its receptors. Also, soluble VEGF receptors with high affinity for VEGF have been designed to prevent VEGF from binding to VEGF receptors on ECs. Furthermore, various small-molecule tyrosine kinase inhibitors (TKIs) have been developed to inhibit VEGF TKRs. Two unique classes of drugs target the mRNA used to code for VEGF. One class is designed to target posttranscriptional modification of mRNA and actually prevents the protein translation of VEGF (37). The other class uses small interfering strands of RNA (siRNA) to prevent the transcription of VEGF mRNA (38).

VEGF inhibition in AMD

Pegaptanib (Macugen®) is approved by the FDA for the treatment of wet AMD. Pegaptanib is an aptamer, an oligonucleotide (short strands of RNA) that assumes a specific three-dimensional shape and binds with high affinity to target molecules. Pegaptanib reduces neovascularization by inhibiting a specific isoform of VEGF, $VEGF_{165}$. Efficacy and safety were evaluated in two randomized, sham-controlled clinical trials. These two combined trials are known as the VEGF Inhibition Study in Ocular Neovascularisation (VISION), which enrolled 1,186 patients. Patients received either an intraocular injection of pegaptanib or a similar sham injection every 6 weeks. Visual acuity was measured using Snellen eye charts, in which patients are asked to identify specific-sized letters or lines at a set distance. Analysis revealed a significant difference in loss of visual acuity compared to sham by 1 year (a loss of 7.93 letters for pegaptanib *versus* 15.05 letters for sham; $p < 0.0001$), which was maintained at 2 years. Also, the risk of severe loss of visual acuity (loss of 30 letters or more) from baseline was 22% in the sham injection group compared to 10% in the group receiving pegaptanib ($p < 0.001$). In addition, patients in the sham group were more likely to lose 3 or more Snellen lines from their vision compared to pegaptanib at 1 and 2 years ($p < 0.001$ at 1 year and $p < 0.05$ at 2 years). These results indicate that pegaptanib is effective in reducing vision loss compared to sham injection in patients with several types of AMD (39, 40).

A cost-effectiveness analysis of the drug was performed in 2005 from the perspective of the U.K. government. This analysis determined that the drug was cost-effective, with a mean incremental cost ratio (ICER) of £8023 per vision year saved, well below the threshold determined to be £20,000 per vision year saved (41).

Ranibizumab (Lucentis®) is also approved for the treatment of wet AMD. Ranibizumab was studied in a 2-year, double-blind, randomized, sham-controlled phase III study. Patients received either low-dose ranibizumab (n=238), high-dose ranibizumab (n=240) or a sham injection given intravitreally monthly for 2 years in 1 eye. The primary outcome of visual acuity was assessed by measuring the number of patients who lost fewer than 15 letters from baseline. Compared to the sham injection group, patients receiving ranibizumab were more likely to lose fewer than 15 letters (94.5% on low-dose ranibizumab, 94.6% on high-dose ranibizumab and 62.2% on sham injection; $p < 0.001$). In fact, vision improvement was noted. The mean visual acuity improved by about 7 letters in the ranibizumab group compared with a decline of 10 letters in the sham injection group ($p < 0.001$). At the study conclusion, 26.1% and 33.3% of patients in the low- and high-dose ranibizumab group, respectively, had a visual acuity gain of 15 letters or more compared with 3.8% of patients in the sham injection group ($p < 0.001$) (41, 42).

Verteporfin photodynamic therapy (PDT) is indicated for wet neovascular AMD and prior to VEGF-inhibiting therapy was the treatment of choice in wet AMD. A recent 2-year, multicenter, double-blind, randomized study compared ranibizumab to verteporfin PDT. Patients received either low or high doses of ranibizumab or verteporfin PDT. Patients receiving ranibizumab had significantly better visual acuity, as indicated by more patients losing fewer than 15 letters on Snellen charts. Also, patients in the ranibizumab group gained 15 or more letters in visual acuity (35.7% on the low dose and 40.3% on the high dose) compared to the verteporfin group (5.6%; $p < 0.001$). Severe loss of visual acuity, indicated by a decline of 30 letters or more, occurred among 13.3% of patients receiving verteporfin compared with none receiving ranibizumab ($p < 0.001$). Two cases of presumed endophthalmitis and 1 case of serious uveitis were reported in the high-dose ranibizumab group, while none occurred in the verteporfin or low-dose ranibizumab group (43).

No trials have been conducted comparing ranibizumab to pegaptanib. However, current data show that ranibizumab actually produces an increase in visual acuity from baseline. To date, trials involving ranibizumab have yet to show increases in visual acuity, only a reduction in further vision loss. There are currently no long-term data to determine if the effects of these therapies are long-lived or if eventually alternative angiogenesis pathways may overcome VEGF inhibition and disease progression will ensue.

Ongoing clinical trials are currently investigating other therapies for AMD that target VEGF. A soluble VEGF receptor, VEGF trap (aflibercept), is being studied in phase III trials as an intravitreal injection. Also, trials examining the systemic and intraocular administration of bevacizumab (Avastin®), a VEGF mAb (see below), to treat AMD are under way. Furthermore, a recent study examined the use of an HIF-1-specific siRNA molecule (38). Data from this study revealed that this siRNA molecule resulted in a marked decrease in VEGF mRNA and protein levels within RPE cells. Consequently, a therapy utilizing siRNA molecules, known as Cand5 (bevasiranib), is being examined in phase II-III trials. Other studies are also under way examining if these AMD therapies may be useful in other ocular disease states such as diabetic retinopathy.

VEGF inhibition in cancer

1. Monoclonal antibodies

Bevacizumab (Avastin®) is a recombinant humanized mAb directed against VEGF. Bevacizumab was designed by inserting VEGF-binding residues (from VEGF receptors Flt-1 and Flk-1) into human immunoglobulin 1 (IgG_1). Bevacizumab is able to bind and neutralize all isoforms of VEGF. It was approved by the FDA in 2004 for first-line combination therapy of metastatic colorectal cancer and has since gained approval for other carcinomas.

Bevacizumab combined with bolus IFL (irinotecan, 5-fluorouracil, leucovorin) and FOLFOX4 (5-fluorouracil, leucovorin, oxaliplatin) is approved by the FDA for first- or second-line therapy in patients with metastatic colorectal cancer. In a phase III trial, the addition of bevacizumab to IFL therapy resulted in increased survival, progression-free survival (PFS), response rate and duration of response compared to bolus IFL alone (44). Results reported for the primary comparison group (bolus IFL/bevacizumab *vs.* bolus IFL/placebo) were as follows: survival 20.3 months *vs.* 15.6 months ($p < 0.001$), PFS 10.6 months *vs.* 6.2 months ($p < 0.001$), response rate 44.8% *vs.* 34.8% ($p = 0.004$) and duration of response 10.4 months *vs.* 7.1 months ($p = 0.001$). Adverse events potentially related to bevacizumab included bleeding, hypertension, proteinuria, gastrointestinal perforation and thromboembolic events.

As a second-line treatment, combination of bevacizumab and FOLFOX4 was shown to be favorable compared to FOLFOX4 alone (45). Data from a phase III clinical study demonstrated that the addition of bevacizumab to regimens improves overall survival (OS) and PFS in patients with advanced or metastatic colorectal cancer previously treated with irinotecan. However, it did not achieve the primary objective of a 40% rate of OS (although there was a significant 16% improvement in OS). The bevacizumab alone arm was terminated early when interim data showed inferior survival data. Adverse events included hypertension, bleeding, vomiting, neuropathy and bowel perforation.

In advanced or metastatic colorectal cancer, third-line combination therapy with bevacizumab and fluorouracil/leucovorin (5-FU/LV) showed low response rates for those patients refractory to oxaliplatin- and irinotecan-based chemotherapy regimens. Median time to tumor progression (TTP) for all treated patients (n=337) was 3.7 months. However, a phase II clinical trial using the combination of cetuximab/bevacizumab (CB) or cetuximab/bevacizumab/irinotecan (CBI) demonstrated favorable

efficacy in patients with irinotecan-refractory metastatic colorectal cancer compared to historical control patients. The TTP was 5.6 months for CB and 7.9 months for CBI and response rates for CB and CBI were 23% and 37%, respectively. These combinations are favorable compared to the historical results for therapies without bevacizumab. Together these trials show mixed results, indicating that further studies are warranted (46, 47).

Bevacizumab is also approved by the FDA for use in combination with carboplatin and paclitaxel for the first-line treatment of unresectable, locally advanced, recurrent or metastatic, non-squamous, non-small cell lung cancer (NSCLC). Data show improved response rates, TTP and OS (48, 49). In a phase II/III trial, the addition of intravenous bevacizumab to paclitaxel and carboplatin significantly improved TTP and median OS compared to the combination regimen of paclitaxel and carboplatin alone in NSCLC. Patients with centrally located tumors close to major blood vessels had an increased rate of major bleeding episodes resulting in death. Mortality data indicate that this combination should be used only in select patient populations and should not be used if patients present with brain metastases, hemoptysis, a history of bleeding or anticoagulation therapy.

Data from a phase II trial in adult patients with metastatic renal cell carcinoma indicate a prolonged TTP in those treated with bevacizumab alone (50). Median TTP increased from 2.5 months in the placebo group to 4.8 months in the high-dose bevacizumab group. Eventual tumor escape from VEGF blockade occurred in most patients (suggested mechanisms for this phenomenon included alternative angiogenesis pathways or insufficient blockade of VEGF by bevacizumab). These results indicate that TTP was significantly prolonged in patients with metastatic renal cancer receiving bevacizumab, although survival was not improved. Common adverse events related to bevacizumab were hypertension and proteinuria. Other adverse events that occurred in 10% or more of patients receiving bevacizumab included epistaxis, fever without infection, malaise, hematuria, hyponatremia and elevated alanine aminotransferase.

In a phase II clinical trial (n=63), combination therapy with erlotinib and bevacizumab produced a response rate of 25% in patients with metastatic or unresectable, locally recurrent clear cell renal cell carcinoma (51). Patients were previously untreated or had received one prior treatment with IL-2 and/or interferon. Previously untreated patients had a longer PFS compared to previously treated patients (12.9 months *vs.* 8.9 months; $p = 0.038$). Toxicities included diarrhea, rash, nausea/vomiting, hypertension, bleeding, proteinuria and pruritus. Results indicate that for patients with metastatic renal cancer, bevacizumab in combination with erlotinib has comparable efficacy to treatment with nephrectomy and interferon alfa-2b. Also, in a phase I/II clinical trial in patients with metastatic renal cell carcinoma, triple therapy with bevacizumab, erlotinib and imatinib produced an overall response rate of 9% and a stable disease/minor response rate of 61% in patients with metastatic or unresectable, locally recurrent clear cell renal cell carcinoma (52). Nine-month PFS and OS were 66% and 70%, respectively. This combination of bevacizumab, erlotinib and imatinib shows no increased efficacy when compared to bevacizumab/erlotinib.

In patients with HER2-negative metastatic breast cancer, bevacizumab in combination with paclitaxel was proven superior to paclitaxel alone (53). A phase III clinical trial comparing paclitaxel plus bevacizumab to paclitaxel alone showed improved PFS. OS was also improved with the addition of bevacizumab, although medians had not yet been reached. Significant adverse events compared to the control group included hypertension, thromboembolic events, bleeding and proteinuria.

Favorable results were reported for patients with advanced pancreatic carcinoma using combination therapy of bevacizumab and gemcitabine in an open-label phase II trial (54). However, a randomized phase III trial (602 patients) had to be terminated early when analysis showed no difference in median survival between gemcitabine plus bevacizumab and gemcitabine plus placebo. It was concluded that the addition of bevacizumab to gemcitabine does not improve survival compared to gemcitabine in advanced pancreatic cancer (55). Adverse events included hematological toxicities similar to single-agent gemcitabine and common bevacizumab toxicities, including hypertension, proteinuria, bleeding complications, visceral perforations and thromboembolic events.

2. Soluble VEGF receptors

Soluble VEGF receptors bind free VEGF, ultimately preventing VEGF receptor binding and activation. VEGF trap (aflibercept) is a modified soluble receptor for VEGF created by fusing binding regions of VEGF receptors with the Fc region of human IgG_1. It binds all forms of VEGF and has higher affinity for VEGF then bevacizumab. *In vitro*, it was shown to completely block phosphorylation of VEGFR-2 when added in 1.5-fold molar excess to VEGF (56). VEGF trap preclinical data in mice have shown decreased tumor growth in various human cancer cell lines, decreased ascites formation with ovarian cancer cell lines and improved tumor control when given in combination with radiation for glioblastomas (56, 57). Phase I clinical trial data showed similar toxicities to other anti-VEGF therapies, including fatigue, proteinuria, hypertension and thromboembolic events (58). Ongoing phase III clinical trials are investigating its use in NSCLC, ovarian and other cancers.

3. Tyrosine kinase inhibitors

Sunitinib (SU-11248, Sutent®) is a TKI that targets multiple TKRs, including PDGFRs, VEGFR-1, VEGFR-2, VEGFR-3, stem cell factor (SCF) receptor, Fms-related tyrosine kinase 3 (FLT3), colony-stimulating factor 1 receptor (CSF-1R) and the glial cell line-derived neurotrophic factor (GDNF) receptor. *In vitro*, inhibition of these TKRs prevents tumor growth, pathological angiogenesis and metastatic progression of cancer (59). Sunitinib is currently indicated by the FDA for gastroin-

testinal stromal tumors (GISTs) and advanced renal cell carcinoma. Compared with placebo, sunitinib improved TTP and PFS in patients with GISTs who had previously not responded to imatinib (60).

Another TKI, sorafenib (Bay-43-9006, Nexavar®), also inhibits tumor angiogenesis by blocking the activation of several TKRs involved in neovascularization and tumor progression, including VEGFR-2, VEGFR-3, PDGFR-B, FLT3, c-Kit and MAPK p38 α. In addition, sorafenib inhibits the activity of Raf-1 and B-Raf, which are involved in the regulation of endothelial apoptosis (61, 62). Sorafenib is approved by the FDA and has been studied in phase III trials for advanced renal cell carcinoma (63). In phase III trials, oral sorafenib prolonged PFS compared with placebo in patients with advanced clear cell renal cell carcinoma in whom first-line therapy had failed. Also, the partial response rate was significantly higher in the sorafenib group compared to placebo. Treatment was associated with increased adverse events, including diarrhea, rash, fatigue, hand-foot skin reactions, hypertension and cardiac ischemia. Sorafenib also significantly increased PFS in patients (n=65) with advanced renal cell carcinoma in a placebo-controlled phase II trial (64).

Sorafenib improved OS rates and TTP in patients with advanced hepatocellular carcinoma in phase II and III trials (65, 66), and FDA approval for this indication was obtained in 2007. No significant differences were found in the overall response rate assessed by Response Evaluation Criteria in Solid Tumors (RECIST) (sorafenib 2.3% *vs.* placebo 0.7%) or time to symptomatic progression (TSP), and no complete responses were observed. Significant differences in OS were consistent across all stratified subgroups, except those with extrahepatic spread. No significant differences in serious adverse events were identified between sorafenib (52%) and placebo (54%). The most common toxicities were diarrhea, hand-foot skin reactions, anorexia, alopecia and nausea. In addition, sorafenib showed favorable activity in the treatment of advanced hepatocellular carcinoma in a phase II clinical.

AEE-788 is a potent combined inhibitor of both epidermal growth factor (EGF) and VEGFR tyrosine kinase family members. *In vitro*, EGF and VEGF receptor phosphorylation was efficiently inhibited and AEE-788 demonstrated antiproliferative activity against a range of EGFR- and erbB-2-overexpressing cell lines and inhibited the proliferation of EGF- and VEGF-stimulated human umbilical vein endothelial cells (HUVECs). *In vivo*, AEE-788 decreased tumor growth in a number of cancer cell lines that overexpress EGFR and or erbB-2. Oral administration of AEE-788 to tumor-bearing mice resulted in high and persistent compound levels in tumor tissue. In addition, AEE-788 also inhibited VEGF-induced angiogenesis in a murine implant model (67). Consequently, AEE-788 is currently being studied in phase I clinical trials.

Axitinib (AG-013736) is a selective oral inhibitor of VEGFR-1, -2 and -3. In a phase II clinical trial (n=52), axitinib was studied in patients diagnosed with metastatic renal cell cancer who had failed on previous cytokine-based treatment. The primary endpoint was objective response (based on RECIST criteria) and secondary endpoints were duration of response, TTP, OS, safety, pharmacokinetics and patient-reported health-related quality of life. Results were recently made available, showing 2 complete and 21 partial responses, with an objective response rate of 44.2% and a median response duration of 23.0 months. Treatment-related adverse events included diarrhea, hypertension, fatigue, nausea and hoarseness. Results indicate that axitinib has clinical activity in patients with cytokine-refractory metastatic renal cell cancer (68).

Cediranib (AZD-2171, Recentin™) is a highly potent ATP-competitive inhibitor of recombinant KDR tyrosine kinase. *In vitro* experiments using HUVECs demonstrated its ability to inhibit VEGF-stimulated proliferation and KDR phosphorylation. In addition, utilizing a fibroblast and endothelial cell model to induce vessel sprouting, cediranib was shown to effectively reduce vessel area, length and branching (69). *In vivo* experiments using human tumor xenografts (colon, lung, prostate, breast and ovary) in mice showed that cediranib was able to inhibit growth in all models (70). A phase I trial investigated cediranib as monotherapy and in combination with gefitinib in patients with advanced renal cell carcinoma (71). Adverse events included hypertension, dysphonia, nausea, vomiting, diarrhea and fatigue. Reductions in plasma levels of VEGFR-2 were observed in renal cell carcinoma patients when cediranib was administered as monotherapy or in combination with gefitinib. Nineteen patients were evaluated for efficacy (RECIST): 2 of 3 monotherapy patients experienced tumor regression, 1 of which was confirmed as a partial response (duration of response: 87 days). In the combination study, 6 of 16 (38%) patients achieved a partial response (median duration: 6 months; 3 of 6 ongoing at data cut-off) and 7 of 16 (44%) patients had stable disease Efficacy and safety results appear favorable for cediranib both as monotherapy and in combination with gefitinib in patients with advanced renal cell carcinoma. Other trials are ongoing.

Vandetanib (ZD-6474, Zactima™) is an orally available inhibitor of VEGFR-2 tyrosine kinase with additional activity against the EGFR tyrosine kinase. In preclinical studies, vandetanib blocked *in vivo* phosphorylation of VEGF and EGF TKRs (72). Vandetanib also prevented the growth of human cancer cell lines in nude mice. However, disappointing results were recently reported from a phase II trial in patients with previously treated metastatic breast cancer (73). Forty-six patients were enrolled and the primary endpoint of objective response was not met (there were no objective responses reported). Diarrhea and rash were reported by 26% of patients. Seven patients in the 300-mg cohort had asymptomatic prolongation of the Q-T_c interval. These results show that vandetanib monotherapy is generally well tolerated but has limited efficacy as monotherapy in patients with refractory metastatic breast cancer.

Vatalanib (PTK-787/ZK-222584) is an oral angiogenesis inhibitor targeting all known VEGFR tyrosine kinas-

es, including VEGFR-1, VEGFR- 2, VEGFR-3, PDGFR and c-Kit protein tyrosine kinase (74). Vatalanib was studied in two phase III trials in colorectal cancer with a primary endpoint of OS (75-77). Secondary endpoints included OS and PFS in patients with high lactate dehydrogenase (LDH > 1.5 x upper limit of normal [ULN]). There was no significant difference in OS. However, PFS was significantly longer in the vatalanib arm. LDH, a marker of poor prognosis in colorectal cancer, was predictive of the outcome in the vatalanib arm (77). When treated with vatalanib, patients with high LDH showed a significant improvement in PFS and in OS (75). Although vatalanib did not meet the primary objective of OS improvement, it did improve PFS significantly in the overall population, and showed strong activity (improved PFS and OS) in patients with high baseline serum LDH. Adverse events included hypertension, diarrhea, dizziness and deep vein thrombosis (DVT; both pulmonary embolism and arterial thromboembolism were seen more commonly in the vatalanib arm than the placebo arm).

Pazopanib (GW-786034, Armala) is a TKI that inhibits VEGFR-1, -2, -3, PDGFR and c-Kit. A phase I study demonstrated activity in various types of advanced solid tumors (78). In a phase II trial, pazopanib resulted in stable disease or partial response in 42% (25 of 60) of patients at 12 weeks (79). Adverse events included hypertension, fatigue, diarrhea, nausea and proteinuria. Surprisingly, no cases of hand-and-foot syndrome were reported and only one case of bleeding occurred. Results appear encouraging and phase II/III trials are under way.

AV-951 (KRN-951) is an oral TKI that is specific for VEGFR-1, -2 and -3. AV-951 potently inhibited VEGF-induced VEGFR-2 phosphorylation in endothelial cells. AV-951 blocked VEGF-dependent, but not VEGF-independent, activation of MAPKs and proliferation of ECs. Following oral administration to rats, AV-951 decreased the microvessel density within tumor xenografts and decreased VEGFR-2 phosphorylation within tumor endothelium. It also inhibited tumor growth in a wide variety of human tumor xenografts, including lung, breast, colon, ovarian, pancreatic and prostate cancer (80). In a phase I clinical trial in 40 patients with advanced solid tumors, AV-951 showed promising results. Notably, of the 9 patients with refractory renal cell carcinoma, all achieved either a partial response or stable disease and 1 patient exhibited a response lasting more than 30 months (81). Phase II trials are currently being conducted.

AMG-706 (motesanib) is an orally bioavailable inhibitor of the VEGFR-1, -2, -3, PDGFR and c-Kit receptors in preclinical models. AMG-706 inhibited human EC proliferation induced by VEGF, but not by bFGF, *in vitro*. In addition, it inhibited vascular permeability induced by VEGF in mice. Oral administration of AMG-706 potently inhibited VEGF-induced angiogenesis in the rat corneal model and induced regression of established A431 xenografts (82). In a phase I trial enrolling 71 patients, the most frequent adverse events were fatigue, diarrhea, nausea and hypertension. Thirty-four (61%) patients had stable disease (at least through 1 month). In this phase I study of patients with advanced refractory solid tumors, AMG-706 was well tolerated and there was evidence of antitumor activity (83, 84). Additional studies of AMG-706 as monotherapy and in combination with various agents are ongoing.

4. Posttranscriptional control

PTC-299 is a novel drug designed to modulate post-transcriptional control of VEGF mRNA. It modifies VEGF mRNA at the 5'- and 3'-untranslated regions. Through these mRNA modifications, PTC-299 selectively inhibits tumor production of VEGF. In *in vitro* studies, PTC-299 inhibited the tumor production of all isoforms of VEGF. PTC-299 was shown to block VEGF synthesis in various tumor types, including breast, cervical, colorectal, gastric, lung, ovarian, pancreatic, prostate and renal cell cancer lines. In animal models, PTC-299 monotherapy reduced VEGF concentrations in tumors and plasma, reduced tumor blood vessel density and inhibited tumor growth. In a phase I study in 52 subjects, interim analysis showed mild adverse events, including headache, dizziness, nausea, vomiting and stomach discomfort (37). No bleeding, clotting, hypertension or proteinuria occurred. In these initial studies, PTC-299 looked promising, with fewer adverse events than other anti-VEGF therapies.

Issues with VEGF inhibitors

Although VEGF inhibitors represent the culmination of decades of research in the treatment of several disease states, numerous issues still need to be addressed before their true benefit can be realized. Measuring the efficacy of VEGF inhibitors is difficult. Although tumor regression has occurred in some cases, angiogenesis inhibitors are not typically cytotoxic; rather, they will most likely result in growth stasis. Some of the current criteria used to define whether a therapy is effective may therefore need to be modified. Tumor mass is likely to be a poor indicator of effective therapy with angiogenesis inhibitors.

Monoclonal antibodies have historically been considered a "magic bullet". Consequently, utilizing mAbs has become a cornerstone in cytokine-targeting therapies. However, cases have been reported where endogenous antibodies target these mAbs, rendering them inactive (85). Therefore, as with any mAb, it is likely that these types of reactions will occur with anti-VEGF antibodies.

In addition, blocking VEGF or its receptors may very well block or potentiate the effects of other ligands. It is likely that these receptors are not specific for VEGF and it is difficult to determine what the long-term effects of blocking VEGF and its receptors may be. In clinical trials, frequent adverse events of most VEGF inhibitors include a marked increase in the rate of thromboembolic events. Cancer itself, even without anti-VEGF therapy, has been shown to increase the risk of these events, and thus it seems that concurrent anticoagulation therapy would be beneficial for many patients. However, other data reveal that bleeding is a common adverse event with these therapies as well. Hopefully, future research can enlighten

Table I: Current and future anti-VEGF agents.

Compound	Status	Other information
Pegaptanib (Macugen®)	Approved	Wet (neovascular) AMD
Ranibizumab (Lucentis®)	Approved	Wet (neovascular) AMD
Bevacizumab (Avastin®)	Approved	First- and second-line metastatic colorectal cancer, first-line NSCLC
Sorafenib (Nexavar®)	Approved	Advanced liver carcinoma
Sunitinib (Sutent®)	Approved	Second-line gastrointestinal stromal tumors, advanced renal cell carcinoma
VEGF trap (aflibercept)	Phase III	High-affinity VEGF binding; similar adverse event profile to other VEGF inhibitors in phase I/II
Axitinib (Recentin™)	Phase II/III	Targets all VEGF receptors; shows activity against renal cell carcinoma
Cediranib	Phase II/III	ATP-competitive TKI; appears effective in renal cell carcinoma in combination with gefitinib
Vandetanib (Zactima™)	Phase II/III	Targets both VEGF and EGF receptors; limited efficacy as monotherapy in metastatic breast cancer
Pazopanib (Armala)	Phase II/III	Targets VEGF receptors; favorable phase I results; no cases of hand-and-foot syndrome, only 1 case of bleeding
AMG-706 (motesanib)	Phase II/III	Targets multiple TKRs; phase I results show activity against several solid tumors
Vatalanib	Phase II	Targets multiple TKRs; improved progression-free survival in colorectal cancer patients, but failed to reach primary endpoint: 40% overall survival rate
AV-951 (KRN-951)	Phase II	Targets VEGF receptors; all patients in a phase I trial had partial response or stable disease
AEE-788	Phase I/II	Targets both VEGF and EGF receptors
PTC-299	Phase I	Acts on posttranscriptional modifications of VEGF mRNA; no bleeding, clotting or proteinuria occurred in a phase I trial

AMD, age-related macular degeneration; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; TKI, tyrosine kinase inhibitors; TKR, tyrosine kinase receptors; EGF, epidermal growth factor.

practitioners as to which patient populations are at risk for an adverse event and therapy can be tailored accordingly.

Beyond VEGF-targeted therapies

VEGF inhibitors are a milestone in drug development; in spite of this, the above-mentioned issues make it unlikely that they will be useful in all patients. VEGF inhibitors appear to be of value in many but not all types of cancer. Trials using VEGF inhibitors, either alone or in combination with chemotherapy, have provided mixed results. For this reason, it would be helpful to have diagnostic testing available to determine which patients would benefit from therapy. Perhaps certain patient populations will be identified that would benefit most by targeting a specific angiogenic growth factor or a specific drug class targeting that growth factor. Moreover, it seems necessary to identify potential antagonism/synergy between certain agents, thus allowing us to predict the most effective combinations and enabling practitioners to overcome redundancies that are built into the angiogenic process. Other novel therapies with different targets may potentially have fewer adverse events and benefit certain populations that cannot receive anti-VEGF. Numerous ongoing trials are focusing on different mechanisms and targeting other regulators of angiogenesis (Table I).

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