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VEGF-targeted cancer therapy strategies: current progress, hurdles and future prospects

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Abstract

Despite setbacks, the clinical development of antiangiogenic agents has accelerated remarkably over the past 3–4 years. Consequently, there are currently three direct inhibitors of the VEGF pathway approved for use in cancer therapy. Other agents that block the VEGF pathway are in advanced stages of clinical development and have shown promising results. With these exciting developments come crucial questions regarding the use of these new molecular-targeted agents, alone or in combination with standard cytotoxic or targeted agents. Importantly, the mechanisms of action of anti-VEGF therapy remain unknown. Here, we discuss several potential mechanisms of action such as tumor vascular normalization, bone marrow-derived cell recruitment blockade and cytostatic effects of anti-VEGF therapy. We review the current progress, the major stumbling blocks and the future directions for anti-cancer therapy using anti-VEGF agents, emphasizing clarification of the underlying molecular mechanisms of action and biomarker identification and validation.

Targeting VEGF for cancer therapy

Vascular endothelial growth factor (VEGF-A or VEGF) (see Glossary) was purified from bovine pituitary follicular cells [1]. The *VEGF* gene encodes the vascular permeability factor (VPF), identified in tumor ascites [2]. VEGF has crucial roles in embryonic development, during which even a slight change in its expression can be lethal [3–5]. In adults, VEGF acts as a potent inducer of vascular permeability and as a survival and mitogen factor for endothelial cells [6,7]. VEGF has important roles in physiological neovascularization during processes such as wound healing or the menstrual cycle. In most cancers, VEGF is overexpressed and is generally associated with disease progression and decreased survival rate [8]. Based on successful randomized Phase III trials, anti-VEGF therapeutics have entered clinical practice for the therapy of cancer in the USA and elsewhere. Bevacizumab (Table 1), a VEGF-specific antibody, was the first antiangiogenic agent to enter the clinic, and is currently approved for use in the USA with standard chemotherapy as the first and second line of treatment in colorectal cancer and as the first line of treatment in lung cancer (<http://www.cancer.gov/cancertopics/factsheet/AvastinFactSheet>). Sorafenib and sunitinib

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(Table 1), two broad-spectrum tyrosine kinase inhibitors (TKIs) that, in addition to inhibiting multiple receptor and soluble kinases in cancer cells, target VEGF receptors in endothelial cells, are also approved for use in the USA. These two agents are recommended as monotherapy in metastatic renal cell carcinoma, and sunitinib is also approved for imatinib-resistant gastrointestinal stromal tumor (GIST) patients. With other anti-VEGF agents in advanced phases of development such as cediranib (AZD2171, Recentin), vandetanib (ZD6474, Zactima), vatalanib (PTK787/ZK222584) and others [8], it is likely that anti-VEGF therapy will soon be widely used for various tumor types. In our opinion, the transient normalization of tumor vasculature by anti-VEGF therapy has a crucial role in tumor response to these therapeutic regimens [9]. This and other mechanisms of action proposed for anti-VEGF therapy [10–12] are currently under intense investigation. Here, we summarize the current progress and point to the potential role of anti-VEGF therapies as treatment for cancer, giving emphasis on the biological processes underlying tumor response to this type of treatment.

Current progress

By currently using anti-VEGF agents, the crucial step of bringing antiangiogenic therapy to the clinic has been made. Bevacizumab was the first agent to be approved by the United States Food and Drug Administration in 2004, and is the most widely utilized anti-VEGF agent to date. Bevacizumab has shown activity (i.e. increased response rates) against most tumors in which it has been tested (e.g. metastatic colorectal, lung and breast tumors) and improves survival in patients with colorectal and lung cancer when administered with standard chemotherapy in randomized placebo-controlled Phase III clinical trials [13,14]. Similarly, the therapy of metastatic renal cancer has been redefined by the successes of two broad-spectrum TKIs that also target VEGF receptors – sunitinib and sorafenib [15,16]. Sunitinib, which targets mutated c-KIT in cancer cells, has produced a significant increase in overall survival of patients affected by imatinib-resistant GIST in a recently completed randomized placebo-controlled trial [17]. Many other Phase III trials of these and other anti-VEGF agents are ongoing or planned on the basis of promising Phase II clinical trial results (e.g. ovarian, renal and brain cancer) and hold great promise in shaping the future of clinical practice in oncology [8].

Current hurdles and future prospects

Because tumor growth and metastasis depend on new vessel development, interfering with angiogenic signals is a logical approach for treatment of solid cancers [18]. VEGF is expressed in most tumors from the early stages of growth [19]. It acts as a mitogen, survival and migration factor for endothelial cells and is a potent inducer of vascular permeability [6,7]. Therefore, it is not surprising that targeting the VEGF pathway has been successful in the clinic. However, randomized Phase III trials of bevacizumab with standard chemotherapy did not improve overall survival as second line treatment in chemorefractory metastatic breast cancer, or as first line treatment in metastatic pancreatic cancer patients. Moreover, three randomized Phase III trials of semaxinib (SU5416) and vatalanib (Table 1) – both anti-VEGF-receptor-selective TKIs – have showed disappointing results when they were combined with standard chemotherapy regimens [8]. Notably, even in successful trials only a subset of patients benefited in terms of survival prolongation when treated by bevacizumab. Furthermore, in these trials the tumor response evaluated by criteria that have been standardized for cytotoxic therapies (response evaluation criteria in solid tumors, RECIST) might not have predicted the utility of anti-VEGF agents, because the latter might not have overt cytotoxic effects.

All these challenges stem primarily from the fact that the mechanism of action of various anti-VEGF agents in cancer patients is incompletely understood [8,11,12,20]. These agents have been developed to halt tumor angiogenesis and destabilize existing tumor vasculature to block the tumor blood supply. However, VEGF blockade might affect survival of certain cancer cells

that express VEGF receptors, and might have also effects on blood circulating progenitors and mature endothelial and hematopoietic cell populations. Despite these crucial functions of VEGF and in spite of promising results in preclinical models and in a Phase II study in renal cancer patients [21], monotherapy with bevacizumab has shown little or no activity in other solid tumors. Therefore, anti-VEGF therapy with bevacizumab has been pursued with standard chemotherapy regimens. Nevertheless, many protocols still include maintenance bevacizumab monotherapy following the completion of combined treatment. Trials are also being carried on to understand the potential role of continuing anti-VEGF therapy after tumor progression. A growing body of preclinical evidence suggests that angiogenic pathways that are distinct from that of VEGF are becoming increasingly important in disease progression or recurrence after anti-VEGF therapy. For example, basic fibroblast growth factor (bFGF, also known as FGF2) is a potent mitogen, migration and survival factor for endothelial cells. In preclinical models, bFGF could drive tumor angiogenesis in a VEGF-independent manner [22,23]. Similarly, stromal-derived factor 1 α (SDF1 α , also known as CXCL12) is a chemokine that can induce tumor neovascularization in mice, alone or with VEGF [24–26]. In recurrent glioblastoma patients treated with cediranib monotherapy in a recent Phase II trial, increased plasma levels of bFGF and SDF1 α correlated with disease progression [27]. These findings warrant further studies to characterize the causal relationship between these well-characterized molecular targets and angiogenesis during tumor relapse despite anti-VEGF therapy. Furthermore, newer molecular candidates, such as the angiopoietins, NOTCH or axon-guiding molecules should be studied in this context. Angiopoietin-1 and angiopoietin-2 bind to their cognate tyrosine kinase receptor TEK/TIE2 on endothelial cells, and modulate endothelial-cell survival and angiogenesis in a context-dependent manner [28,29]. Recently, the NOTCH family has been intensely studied because of their emerging role in tumor angiogenesis in close interrelation with VEGF [30–32]. Finally, it has been recently recognized that the four major classes of axon-guidance ligands (ephrins, semaphorin, slits and netrins) and their receptors have crucial roles in vessel guidance. The demonstration that vascular and neural morphogenesis shares many more processes than originally thought has opened new research avenues for antiangiogenesis in tumors [33,34].

The increased overall survival in advanced-stage cancers has provided crucial proof-of-principle validation for the clinical efficacy of anti-VEGF therapy. However, the absolute gain in overall survival remains 2–5 months and is seen only in certain cancers (colorectal and lung) but not others (pancreatic and breast) [13,14,35]. One reason might be that the optimal dose of anti-VEGF agents and treatment scheduling are not yet clearly defined for combined regimens for cancer patients. Integration of correlative studies to address this question in large national or multinational randomized trials has been difficult. In addition, a validated biomarker for activity of these targeted agents has not been identified. When correlative studies to identify predictive markers of response have been performed, no correlation between response and the various tissue and plasma markers analyzed has been identified [8,36,37].

Mechanisms of action

Several mechanisms of action have been proposed for VEGF-blocking agents in cancer patients [8,10,12]. The potential pleiotropic effects of anti-VEGF therapy is in part due to the fact that VEGF receptors are expressed not only on endothelial cells in tumors, but also on subsets of hematopoietic cells, stromal cells, in addition to malignant cells in certain human cancers (e.g. breast, brain, colorectal and pancreatic [11,38–40]). The existing clinical data on the mechanisms of action of anti-VEGF therapy that have been suggested by preclinical studies [9,11,12] are discussed below. In terms of direct anti-vascular effects, a wealth of preclinical data has demonstrated that anti-VEGF agents alone can block angiogenesis and regress tumors or delay tumor progression [41,42]. Recent studies have proposed an ‘anti-vasculogenic’ effect of VEGF blockers, manifested through the blockade of blood-borne endothelial precursor cell

recruitment in tumors [43]. Finally, based on preclinical observations, it has been suggested that anti-VEGF agents can ‘normalize’ tumor vasculature [9,44] (Figure 1).

A comprehensive correlative study tested the normalization hypothesis in a Phase I–II trial of bevacizumab in locally advanced rectal cancer patients. The treatment regimen consisted of an initial bevacizumab infusion followed by three cycles of bevacizumab, 5-fluorouracil (5-FU) and external beam local radiotherapy. Sixto-nine weeks after completion of the combined treatment, patients underwent resection of the tumor. Using multiple functional, cellular and molecular investigations, direct evidence was obtained for the antivascular effect of bevacizumab in rectal cancer patients [45]. Bevacizumab reduced the tumor vascular density by ~50% at day 12 after the first infusion, significantly reduced the tumor blood flow [evaluated from computer tomography (CT) scans] and the number of viable circulating endothelial cells (CECs) and progenitor cells (CPCs). The tumors in both the low-dose [45] and the high-dose bevacizumab [46] infusion groups had a less hyperemic or hemorrhagic appearance. However, there was no significant tumor regression after bevacizumab treatment, as determined by flexible sigmoidoscopy examination, which enables macroscopic visualization of the rectal cancer. The reduction in tumor vasculature led to a significant increase in cancer cell apoptosis. Interestingly, it also led to the emergence of a more mature (pericyte-covered) vasculature [45,46] and increased cancer cell proliferation [46] (Figure 2). Twelve days after bevacizumab therapy alone, the tumor interstitial fluid pressure (IFP) was consistently decreased, particularly in patients with high baseline values [45,46]. This suggests that in human tumors, similar to mouse models [47,48], the tumor microenvironment is normalized by the reduction of the excess abnormal vasculature and is potentially sensitized to subsequent cytotoxic therapy.

Imaging studies also provided supportive data for the normalization hypothesis. Fluorodeoxyglucose (FDG)-positron emission tomography (PET) detects metabolically active malignant lesions, and can be used to stage and monitor tumor response to treatment. Despite the significant reduction in vessel density and blood flow, FDG-uptake measured from PET scans did not significantly change at day 12. Moreover, despite the decrease in vessel surface area (S) and blood flow, the permeability–surface area product ($P \cdot S$) (proportional to the penetration of tracer in the tumor), evaluated from perfusion CT scans, did not significantly change at day 12 [45,46]. The changes observed in these two parameters suggest that the tumor vasculature becomes more efficient after anti-VEGF treatment in some areas of the tumors. However, whether the increase in tumor cell apoptosis is due to a direct effect of bevacizumab or an indirect effect due to insufficient perfusion in certain areas is unclear. If bevacizumab is acting in concert with chemotherapy as a cytotoxic agent for endothelial and/or cancer cells, it might be expected that using higher doses of the antibody should produce increased efficacy. However, a lower dose of bevacizumab (5 mg/kg) was found to be more effective than the higher dose (10 mg/kg) with standard chemotherapy as first line of treatment in metastatic colorectal cancer patients in a Phase II trial [49] and, subsequently, in the pivotal Phase III trial [13]. In previously untreated, advanced non-squamous, non-small cell lung cancer (NSCLC), although a dose of 15 mg/kg bevacizumab every three weeks increased overall survival with chemotherapy [14], a more-recent international Phase III trial (the AVAiL study) indicated that 7.5 mg/kg bevacizumab was equally effective (<http://www.gene.com/gene/news/index.jsp>). This result supports the idea of a bell-shaped curve for the dose of bevacizumab. Accordingly, the ‘low doses’ of bevacizumab tested in these trials might represent the optimal biological doses for these indications. Therefore, they might represent the ‘normalizing dose’ for bevacizumab in the combined bevacizumab–chemotherapy regimen and/or the ‘optimal angiostatic dose’ for bevacizumab monotherapy after the completion of the combined treatment (see trial design and discussion in [14]). Finally, ‘high doses’ (10 mg/kg) of bevacizumab did not prolong overall survival with chemotherapy in breast and pancreatic cancers, which express functional VEGF receptors.

Similarly, the role of VEGF blockade on immune or stromal cells in patients is not yet understood. However, preclinical data suggest a potential role for at least some of the bone-marrow-derived cell populations that migrate to tumors during or after therapy [12]. Interestingly, blood CPCs (defined as CD133⁺CD34^{bright}CD45^{dim} cells) are transiently decreased in number by bevacizumab treatment in rectal cancer patients [45,46,50], and CPC elevation is correlated with the relapse of glioblastomas after cediranib treatment interruption [51]. Subsets of CPCs might express not only VEGF receptors, but also c-KIT and platelet-derived growth factor (PDGF) receptors [43,52], which are also key targets of many of the anti-VEGF receptor TKI agents.

Biomarkers of anti-VEGF therapy

At this time, the identification and validation of a biomarker for this novel type of therapy has eluded investigators. Unlike preclinical models, Phase III studies with bevacizumab in metastatic colorectal cancer patients were not successful in identifying specific molecular signatures – mutation status of p53, K-RAS or B-RAF oncogenes, or protein expression of p53, VEGF or thrombospondin-2 (TSP2) – or microvascular density at baseline as predictive markers of response [36,37] (Table 2).

Some of the most promising biomarkers for antiangiogenic therapy are the blood CECs [53]. Studies in rectal cancer patients have demonstrated that bevacizumab decreased tumor microvascular density and the number of viable CECs (identified phenotypically as CD31^{bright}CD34⁺CD45[−]ToPro3[−] mononuclear cells by flow cytometry), consistent with an antivascular effect [45,46]. Whether these changes have predictive value for the combined bevacizumab–chemoradiation regimen is currently under investigation in a Phase II trial, which is ongoing at Duke University Medical Center and Massachusetts General Hospital (ClinicalTrials.gov Identifier: NCT00052559). In glioblastoma patients, viable CEC numbers increased significantly in parallel with tumor volume at four time points (days 1, 28, 56 and 112) during cediranib monotherapy. Moreover, viable CEC levels (evaluated at eight time points during treatment) were significantly higher in patients who experienced disease progression during cediranib therapy compared with patients without progression at day 112 [51]. No such correlation was observed for CPCs. Rather, as mentioned above, a significant rise in CPCs was observed during glioblastoma relapse after drug interruptions [51]. These findings suggest a differential role of CPCs versus viable CECs in the assessment of clinical outcomes. These data also emphasize the importance of using multiple markers to identify these distinct populations and differentiate them from other blood circulating cells, such as non-viable CECs [54] (probably shed by the tumor endothelium during therapy) or mature hematopoietic cells [55]. They also highlight the need for further studies of the biology of CECs and CPCs.

Plasma angiogenic proteins have also been investigated in multiple Phase I–II and in some Phase III trials. Plasma levels of VEGF and placental-derived growth factor (PlGF, a VEGF family member that specifically binds to VEGF receptor 1) are significantly increased in rectal cancer patients receiving bevacizumab [46]. In apparent contrast to some preclinical findings [56], other groups have reported similar observations in cancer patients treated with various anti-VEGF receptor TKIs (e.g. vatalanib and sunitinib), and have also found a decrease in soluble VEGF receptor 2 levels in the plasma [57–59]. Recently, significant elevations in plasma VEGF and PlGF have been confirmed, together with a decrease in soluble VEGF receptor 2 in glioblastoma patients treated with the anti-VEGF receptor TKI cediranib [51]. Collectively, these clinical correlative data suggest a potential pharmacokinetic and/or pharmacodynamic biomarker value for these three plasma markers and warrant further clinical studies. Another group reported that baseline soluble intercellular adhesion molecule-1 (sICAM1, also known as CD54) levels in the plasma predict response to bevacizumab with

chemotherapy in metastatic lung cancer patients [60]. To date, the source of the soluble forms of these receptors (i.e. VEGF receptor 1 and 2 and sICAM1) and the pathophysiological significance of their presence in the plasma are unclear.

It would be of great value to identify biomarkers that predict disease progression during anti-VEGF therapy. Although in rectal cancer patients significant changes in bFGF have not been identified, data from a recently completed Phase II trial of cediranib in recurrent glioblastoma patients demonstrated a significant correlation between elevation of bFGF and SDF1 α levels and tumor progression [51]. These differences might reflect the prolonged clinical response in rectal cancer patients compared with that of glioblastoma patients, or might be due to disease or agent specificity. Nevertheless, with the development of improved flow cytometry and protein array analysis techniques and subsequent standardization [46,51,61], circulating cell and plasma protein measurements hold promise for identification of valid biomarkers for anti-VEGF therapy.

Toxicity

In the trials that have been completed to date, anti-VEGF therapy has shown mild toxicity. In cancer patients, anti-VEGF agents might induce fatigue, proteinuria, hand and foot syndrome and hypertension, which is manageable by medication. Rare agent-specific toxicities have been reported (e.g. arterial thromboembolic events and reversible posterior leukoencephalopathy for bevacizumab) [10]. Serious complications such as gastrointestinal perforations, hemorrhage or congestive heart failure have been observed in rare cases with bevacizumab in colorectal, lung (particularly in squamous cell type), brain, ovarian and pancreatic cancer. To date, the etiology of all these side effects is unclear. The dose dependence and the long-term effects of anti-VEGF therapies in patients with less advanced-stage cancers remain to be established in large trials [10]. Interestingly, in mice it has been shown that, in 11 of the 17 healthy organs studied, sustained VEGF blockade can significantly but reversibly decrease the number of normal capillaries [62].

Concluding remarks

As clinical development with anti-VEGF agents advances rapidly, two major areas of research are needed in the immediate future. First, to understand the mechanisms of action of anti-VEGF agents further and, second, to identify and validate biomarkers for anti-VEGF therapy (Box 1). Achieving this would enable optimization of different treatment protocols, establishment of appropriate tumor response criteria and, possibly, reduction of the adverse effects. It would also provide a better understanding of the role of anti-VEGF therapy in the adjuvant, neo-adjuvant or maintenance therapy settings.

One mechanism that our group is actively investigating is that of vascular normalization. In addition to improving the delivery and efficacy of many cytotoxic agents, the fortification of blood vessels during normalization might also reduce tumor-associated edema and metastasis [63]. The identification of a vascular normalization time window in patients with specific types of cancer might enable synergistic combinations with chemotherapeutics or radiotherapy. It will be crucial to establish the efficacy of TKIs in combination with cytotoxic therapies. Similarly, a better understanding of the mechanisms of vessel pruning and cancer cell apoptosis induced by anti-VEGF therapy, and tumor escape, might enable further sensitization of tumor cells to cytotoxic therapies. In addition, characterization of the effect of anti-VEGF therapy on the contribution of bone marrow-derived cells to tumor growth and relapse would enable more rational and effective approaches to therapy. Finally, identifying molecular mechanisms involving bone marrow-derived cells or the stromal and interstitial matrix compartments would contribute to establishing more complex and, potentially, more efficacious combinatorial strategies. Finally, identifying mechanisms of relapse through anti-VEGF therapy might

provide clues on how to improve targeting of tumor vessels and extend the survival advantage conferred by VEGF blockade.

Box 1. Outstanding questions

- Should anti-VEGF agents be combined with traditional chemotherapeutics and/or radiation treatment, or with other targeted therapeutics, for optimized efficacy and reduced adverse effects?
- Is there a role for anti-VEGF therapy in the adjuvant or neo-adjuvant or maintenance setting?
- Should anti-VEGF therapy be stopped upon disease progression?
- Can orally available, VEGF-receptor tyrosine kinase inhibitors be used to prolong overall survival in combination with cytotoxic regimens?
- What are the most appropriate methods or criteria for assessing tumor response to anti-VEGF agents?
- What surrogate markers or biomarkers should be used to evaluate and predict response to different anti-VEGF agents?
- What are the molecular mechanisms of tumor relapse after anti- VEGF treatment, or resistance to anti-VEGF therapy, and how might it be possible to delay or prevent this escape?
- What is the importance of targeting mesenchymal and hematopoietic (bone-marrow-derived) cell populations in tumors with anti-VEGF agents?

Collectively, these correlative studies have the potential to clarify some of the discrepancies observed between preclinical and clinical data and, more importantly, might enable an optimized use of antiangiogenic therapy for cancer.

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Glossary

Basic fibroblast growth factor (bFGF or FGF2)

a cytokine that belongs to a homologous family of at least 18 proteins. bFGF binds to FGF receptors 1 and 2, acts as a potent mitogen for endothelial cells, and is expressed in many benign and malignant tumors

Circulating endothelial cells (CECs)

cell populations circulating in the peripheral blood that exhibit an endothelial cell-specific surface marker phenotype (e.g. positive for CD31 and CD34 but not for CD45, a pan-hematopoietic marker)

Circulating progenitor cells (CPCs)

hematopoietic cells populations circulating in the peripheral blood that express progenitor and stem cell-specific surface markers (e.g. CD34 and CD133)

Neovascularization

the generic name for the process of new vessel formation, which encompasses angiogenesis (from pre-existing vessels) and vasculogenesis (from endothelial precursor cells)

Placental-derived growth factor (PlGF)

a family of pro-angiogenic cytokines that belongs to the VEGF family of growth factors. PlGF is expressed in many cancers and specifically binds to VEGF receptor 1, which can be found on endothelial, hematopoietic, mesenchymal or neoplastic cells in tumor stroma

Platelet-derived growth factor (PDGF)

a family of cytokines expressed in many cancers. PDGF binds to PDGF receptors present on mesenchymal, endothelial, hematopoietic or neoplastic cells in tumor stroma

Stromal-derived factor 1 α (SDF1 α or CXCL12)

a chemokine recently discovered to have important roles in neovascularization. SDF1 α binds to CXCR4, which is present on endothelial, hematopoietic or neoplastic cells in tumor stroma

Tyrosine kinase inhibitor (TKI)

a small molecule with tyrosine kinase inhibitory activity for receptor and/or cytoplasmic kinases

Vascular endothelial growth factor A (VEGF-A or VEGF)

a cytokine that is considered crucial for both pathological and physiological angiogenesis. It acts as a surviving, migration and proliferation factor for endothelial cells and as a potent vascular permeability factor. VEGF binds to VEGF receptors 1 and 2 and neuropilin 1, which are present on multiple cell types

Vascular normalization

the process whereby antiangiogenesis prunes and remodels the abnormal tumor vessels, which become closer to normal tissue vasculature in terms of structure and function

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Figure 1.

Tumor vascular normalization. **(a)** Antiangiogenic regimens are aimed to kill most of the vessels and starve the tumor (last column); by contrast, dosing and scheduling of the antiangiogenesis agents might be planned to transiently 'normalize' the vasculature, making it more efficient for drug and oxygen delivery. **(b)** Dynamics of vascular normalization induced by VEGF-receptor-2 blockade. On the left is a two-photon image showing normal blood vessels in skeletal muscle; subsequent images show human colon carcinoma vasculature in mice at day 0, day 3 and day 5 after administration of VEGF-receptor-2-specific antibody. **(c)** Diagram depicting the concomitant changes in pericyte (red) and basement membrane (blue) coverage during vascular normalization. **(d)** These phenotypic changes in the vasculature might reflect changes in the balance of proangiogenic and antiangiogenic factors in the tissue. Reproduced, with permission, from [9].

Figure 2.

Rectal tumor response to VEGF blockade evaluated by immunohistochemistry in serial biopsies. Tumor microvascular density was determined by counting CD31-positive vessels in areas of frank carcinoma. The fraction of proliferating (left plot) and apoptotic (right plot) tumor cell was determined in sections stained for proliferation cell-nuclear antigen (PCNA) and TUNEL, respectively. Note that despite significant reduction in microvascular density, there was an increase in tumor cell apoptosis, and cell proliferation tended to increase 12 days after treatment (Tx) with bevacizumab [45,46].

Table 1
Anti-VEGF therapeutics approved for use in cancer patients

Anti-VEGF targeted agent	Nature of targeting proven efficacy	Approved use	Manufacturer	Refs
Bevacizumab (Avastin®)	VEGF-specific antibody. With standard chemotherapy, it increases overall survival in colorectal and lung cancer and progression-free survival ^a in breast and renal cancer.	First and second line treatment in metastatic colorectal cancer with infusional 5-FU-based combination regimens (USA). First line treatment of patients with locally advanced, metastatic or recurrent non-small cell lung cancer in combination with platinum-based chemotherapy (USA).	Genentech Inc. (South San Francisco, CA)	[13,14]
Sorafenib (BAY 43-9006, Nexavar®)	Broad-spectrum tyrosine kinase inhibitor. It targets VEGF receptors in endothelial cells and increases progression-free survival in renal cancer patients. It also increases overall survival in liver cancer patients ^b .	Advanced renal cell carcinoma.	Bayer AG (Leverkusen, Germany) and Onyx Pharmaceuticals (Emeryville, CA)	[15]
Sunitinib (SU11248, Sutent®)	Broad-spectrum tyrosine kinase inhibitor. It targets VEGF receptors in endothelial cells and increases progression-free survival in renal cancer patients. It also increases overall survival in gastrointestinal stromal tumors.	Advanced renal cell carcinoma; imatinib mesylate-resistant gastrointestinal stromal tumors.	Pfizer (New York, NY)	[16,17]

^a Bevacizumab increases progression-free survival in previously untreated metastatic breast cancer patients (with paclitaxel) and in previously untreated advanced renal cell cancer (with α -interferon) in placebo-controlled randomized Phase III trials (see <http://www.gene.com/gene/news/index.jsp>).

^b Based on a planned interim analysis of data from a placebo-controlled randomized Phase III trial of sorafenib in patients with advanced hepatocellular carcinoma or primary liver cancer, an independent data monitoring committee found that the trial met its primary endpoint, resulting in superior overall survival in those patients receiving sorafenib (see http://www.onyx-pharm.com/wt/page/press_releases).

Table 2

Potential biomarkers currently evaluated for anti-VEGF therapy in cancer patients

Type of evaluation		Marker	Current progress and limitations	Refs
Tumor-tissue analyses	Protein and genomic analyses of tumor tissue	Microvascular density (MVD); protein expression; rate of cell proliferation and/or apoptosis; genetic mutation.	Bevacizumab can decrease MVD and increase perivascular cell coverage. Bevacizumab can increase apoptosis and proliferation. MVD, K-RAS, BRAF and p53 mutation, and p53, VEGF or TSP2 expression at baseline do not predict tumor response to bevacizumab with chemotherapy.	[36,37,40,45,46]
	Interstitial fluid pressure (IFP)	Interstitial fluid pressure (IFP).	Bevacizumab decreases IFP in rectal cancer patients.	[45,46]
	Interstitial oxygen tension	Tissue oxygenation.	No data available.	[64]
Cellular and molecular markers in bodily fluids	Blood circulating cells	Number of circulating endothelial cells (CECs); number of circulating progenitor cells (CPCs).	Bevacizumab decreases viable CECs in rectal cancer patients. Change in CECs predicts progression-free survival in patients with metastatic breast cancer receiving bevacizumab with letrozole. Viable CECs correlate with glioblastoma recurrent growth on AZD2171 treatment. CPCs correlate with glioblastoma relapse after AZD2171 treatment.	[45,46,51,65]
	Protein levels in plasma, serum, urine, ascites and pleural effusions	Soluble protein concentration.	VEGF, PIGF increase after treatment with bevacizumab, sunitinib and cediranib. sVEGFR2 decreases after treatment with sunitinib and cediranib. sICAM1 level at baseline predicts response to bevacizumab with chemotherapy in lung cancer patients. Increased SDF1 α and bFGF levels correlate with	[46,51,57–59]

Type of evaluation		Marker	Current progress and limitations	Refs
Imaging	Computed tomography, positron emission tomography and magnetic resonance imaging	Blood flow and volume; permeability surface area product; mean transit time; tracer uptake; tissue oxygenation.	recurrent glioblastoma progression in patients on cediranib. Blood flow and volume decreased by bevacizumab in rectal and breast cancer. FDG uptake is unchanged by bevacizumab alone, but significantly decreased by bevacizumab with chemoradiation in rectal cancer. Bevacizumab, vatalanib, cediranib and AG-013736 decrease blood flow and permeability. Cediranib decreases relative vessel size. Bevacizumab and cediranib reduce peritumor edema.	[40,45,46,51,66–69]