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Antiangiogenics: The Potential Role of Integrating This Novel Treatment Modality With Chemoradiation for Solid Cancers

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Abstract

Although still in very early stages of clinical development, the combination of antiangiogenics with contemporary chemoradiotherapy regimens has emerged as a feasible and promising approach to many cancers. We review the rationale and the current understanding of antiangiogenics and their therapeutic potential in combination with chemoradiotherapy. Finally, we offer a perspective on future research directions aimed at making this complex therapeutic approach successful in the clinic.

INTRODUCTION

Some of the most promising anticancer therapy approaches tested in recent clinical studies have been combinations of new, molecularly targeted agents with standard therapies. Of these targeted agents, antiangiogenics have been used with chemotherapy in metastatic disease and have shown increases in survival in some of these advanced tumors. In this review, we present an update on the clinical use of antiangiogenic agents, their potential mechanisms of action, and methods of evaluating their effects. The emphasis is placed on the potential use of these agents with chemoradiotherapy in certain cancers.

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ANTIANGIOGENIC APPROACH FOR CANCER TREATMENT

New vessel formation is a critical step in tumor progression from in situ lesions to extensive and distant disease. Proposed in 1971 by Judah Folkman, MD,¹ and later confirmed experimentally in multiple preclinical models, targeting of tumor blood vessels has become an attractive anticancer strategy. Unfortunately, dozens of phase III trials using nonspecific inhibitors of angiogenesis failed to show a survival advantage. The failure of the targeted agent bevacizumab (Avastin; Genentech, South San Francisco, CA), a vascular endothelial growth factor (VEGF)–specific antibody, to increase survival with chemotherapy in previously treated and refractory metastatic breast cancer in a phase III trial² sent many researchers back to the bench in an attempt to clarify the discrepancy between clinical and preclinical results. Conversely, the last 5 years have brought spectacular successes in the clinic for antiangiogenics. These breakthroughs have come with the use of bevacizumab with standard chemotherapy in randomized phase III trials in metastatic colorectal and lung cancer, and with multitargeted anticancer agents that also block VEGF signaling (sorafenib [Nexavar]; Bayer AG, Leverkusen, Germany, and Onyx Pharmaceuticals, Emeryville, CA; and sunitinib [Sutent]; Pfizer, New York, NY) in metastatic renal cell carcinoma, and GI stromal tumors.^{3–7} The United States Food and Drug Administration has approved these three agents for these indications in cancer patients. More promising data come from unpublished phase III data for bevacizumab with paclitaxel in first-line metastatic breast cancer, bevacizumab with interferon alfa-2a in first-line metastatic renal cell cancer, and sorafenib in advanced hepatocellular carcinoma. The fact that anti-VEGF therapy has been brought to fruition in the clinic by complex anticancer and antivascular targeting strategies offers great hope that these and other antiangiogenics could be used with chemoradiotherapy to improve treatment outcomes significantly in certain cancers. Several trials addressing this issue are already underway.

WHY COMBINE ANTIANGIOGENESIS WITH CHEMORADIOOTHERAPY?

More than half of all cancer patients ultimately receive radiation therapy, but their tumors relapse in most cases. In certain disease types, chemoradiotherapy is a standard of care (brain, head-and-neck, anal canal, cervix, and lung cancers). In addition to neoplastic cells, both radiation and chemotherapy have been shown to kill proliferating endothelial cells. In principle, therapy with antiangiogenics may—in addition to preventing new vessel formation—sensitize the endothelial cells to the effect of cytotoxic therapies. This effect could be mediated either by direct blockade of proangiogenic molecules that increase endothelial cell survival (eg, VEGF), or indirectly by interference with the recruitment by angiogenic factors of bone marrow-derived cells to tumor tissue for revascularization. Preclinical evidence has been reported in support of these concepts.^{8,9}

However, the killing of all neoplastic cells requires an adequate blood supply to allow delivery of the agents and maintain tissue oxygenation (a known radiosensitizer). Antiangiogenics have the potential to increase tumor hypoxia and make tumor vessels inefficient for subsequent drug delivery.^{10–12} Yet, antiangiogenics have been successful in potentiating the effects of chemotherapy or radiation therapy in multiple preclinical studies and clinical trials.^{5,7,13–20} They have also been shown to decrease hypoxia in some preclinical models.^{13,19} All of these apparent paradoxical findings could be explained by the transient tumor vascular normalization effect of antiangiogenics.^{21,22}

To obtain nutrients for their growth and to metastasize to distant organs, cancer cells co-opt host vessels, sprout new vessels from existing ones (angiogenesis), and/or recruit endothelial cells from the bone marrow (postnatal vasculogenesis).^{1,23} The resulting vasculature is structurally and functionally abnormal (Table 1).²⁴ These structural abnormalities contribute to spatial and temporal heterogeneity in tumor blood flow. In addition, solid pressure generated

by proliferating cancer cells compresses intratumoral blood and lymphatic vessels, which further impairs not only the blood but also lymphatic flow.²⁵ Collectively, these vascular abnormalities lead to an abnormal tumor microenvironment characterized by interstitial hypertension (elevated hydrostatic pressure outside the blood vessels), hypoxia, and acidosis.

Impaired blood supply and high interstitial fluid pressure interfere with the delivery of therapeutics to solid tumors. Hypoxia renders tumor cells resistant to both radiation and several cytotoxic drugs. In addition, independent of these effects, hypoxia also induces genetic instability and selects for more malignant cells with increased metastatic potential. Finally, hypoxia and low pH compromise the functions of cytotoxic immune cells. Unfortunately, cancer cells are able to survive in this abnormal microenvironment. Interstitial hypertension forces the fluid from the tumor margin to the surrounding tissue (or fluid), contributing to the tumor-associated edema and lymphatic metastasis.²⁶ Thus, the abnormal vasculature of tumors and the resulting abnormal microenvironment together pose a formidable barrier to the delivery and efficacy of chemoradiotherapy. This suggests that if we knew how to correct the structure and function of tumor vessels, we would have an opportunity to normalize the tumor microenvironment and ultimately to improve cancer treatment by chemoradiotherapy. The fortified tumor vasculature may also inhibit the shedding of cancer cells into the circulation—a prerequisite for metastasis.

In normal tissues, the collective action of angiogenic stimulators (eg, VEGF) is counterbalanced by the collective action of angiogenic inhibitors such as thrombospondin-1 (Fig 1). This balance tips in favor of the stimulators in both pathologic and physiologic angiogenesis. However, in pathologic angiogenesis, the imbalance persists. Therefore, restoring the balance may render the tumor vasculature close to normal. Conversely, tipping this balance in favor of inhibitors may lead to vascular regression, and ultimately, to tumor regression. Unfortunately, current antiangiogenics are unable to maintain vascular regression durably, as evidenced by clinically observed inevitable tumor relapse and a return to abnormal new vessel formation, most probably via pathways independent of VEGF.

On the basis of current knowledge, VEGF seems to be the most critical angiogenic molecule in cancer. VEGF promotes the survival and proliferation of endothelial cells, and increases vascular permeability.²⁷ Thus, if one were to judiciously downregulate VEGF signaling in tumors, then the vasculature might revert back to a more normal state. Indeed, blockade of VEGF signaling may passively prune the immature and leaky vessels (ie, have an antivascular effect) and actively remodel the remaining vasculature so that it more closely resembles the normal vasculature (Fig 1).²⁸ This normalized tumor vasculature would be characterized by less leaky, less dilated, and less tortuous vessels with a more normal basement membrane and greater coverage by pericytes (Fig 1). These morphologic changes could be accompanied by functional changes: decreased interstitial fluid pressure, increased tumor oxygenation, and improved penetration of drugs in these tumors (Table 1).

Preclinical and clinical findings (detailed in the following section) support the concept of vascular normalization and its transient nature during treatment with antiangiogenics.^{16–19, 28–35} This has significant implications for the use of antiangiogenics with chemoradiotherapy.³⁶ Early evidence demonstrated that this combined approach is safe and promising in the neoadjuvant setting in locally advanced rectal cancer.^{28,37}

Finally, antiangiogenics have been used in an adjuvant setting and may play important roles, which remain to be established in ongoing studies.³⁸ Of particular interest, recent trials of antiangiogenics in glioblastoma have shown that antiangiogenics can control brain vasogenic edema,^{35,39} and that they may play a role in reducing radiation-induced damage to the brain.⁴⁰

CURRENT PROGRESS ON THE COMBINED OF ANTIANGIOGENICS WITH CHEMORADIO THERAPY

Clinical development of antiangiogenics with chemoradiotherapy is still in its early stages. Several published phase I and II trials have established that addition of antiangiogenics to chemoradiotherapy has not increased the acute toxicity of these regimens in glioblastoma, and rectal and pancreatic cancer.^{37,41,42} Approximately 20 other phase I or II trials registered at the National Institutes of Health are active, and the vast majority of these are exploring combinations of bevacizumab with standard chemoradiotherapy in advanced lung, colorectal, cervical, head-and-neck, esophageal, and pancreatic cancer. Several trials are exploring anti-VEGF receptor tyrosine kinase inhibitors (eg, PTK787 [vatalanib]; Novartis, Basel, Switzerland), ZD6474 (vandetanib [Zactima]; AstraZeneca Pharmaceuticals, Cheshire, United Kingdom), or sorafenib, with chemoradiotherapy in glioblastoma, head-and-neck and pancreatic cancer patients. These studies are expected to provide critical insights into the safety and efficacy of these combinations. It is our firm belief that integrating comprehensive correlative studies in such trials will provide additional mechanistic insights and biomarker exploration.

To test the hypothesis that inhibition of VEGF is safe and results in clinical benefit and enhancement of chemoradiotherapy therapy response, we initiated a phase I/II trial of neoadjuvant bevacizumab in combination with fluorouracil (FU) and radiation therapy in patients with locally advanced T3 or T4 rectal cancer.^{28,37,45} Bevacizumab was delivered on day 1 of each cycle. The dose was escalated in successive cohorts of six patients, beginning at 5 mg/kg followed by 10 mg/kg. Infusional FU was administered during 24 hours each day at a fixed dose of 225 mg/m² throughout each treatment week of cycles 2 to 4. External-beam irradiation was administered during cycles 2 to 4 for a total of dose of 50.4 Gy in 28 fractions during 5.5 weeks. The primary clinical objective of the phase I study was to determine the maximum-tolerated dose of bevacizumab when delivered concurrently with standard chemoradiotherapy in rectal cancer patients before surgery. In parallel, a major goal of this study was to clarify through correlative studies the mechanisms by which bevacizumab inhibits angiogenesis and improves the outcome of other therapeutic modalities in the treatment of this malignancy.

The first six patients treated with the combination of bevacizumab at the 5 mg/kg dose level with chemoradiotherapy tolerated this treatment without difficulty. All six patients underwent surgery without complication. In contrast, two of five patients in the second cohort who were administered high-dose bevacizumab (10 mg/kg) with chemoradiotherapy experienced grade 3 to 4 dose-limiting toxicity of diarrhea and colitis during the combined treatment. After recovery from toxicity, these patients were able to resume and complete radiation therapy and FU treatment. Because of these dose-limiting toxicities, only five patients were enrolled at the 10 mg/kg dose and the maximum-tolerated dose for the phase II trial was set at 5 mg/kg. At surgery, the tumors of the 11 phase I patients usually showed minimal residual disease consistent with Mandard grade 1 to 2 regression. Of interest, pathologic evaluation of the surgical specimens for staging after completion of all therapy showed two complete pathologic responses (Fig 2).

The study design of this trial permitted a unique opportunity to evaluate the effect of bevacizumab alone (cycle 1) on rectal cancer before and after its concurrent administration (cycles 2 to 4) with chemoradiotherapy. Twelve days after the first bevacizumab infusion, patients underwent repeat flexible sigmoidoscopy with tumor biopsy, tumor interstitial pressure measurement, perfusion computed tomography (CT) scan to measure blood flow, [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG) positron emission tomography (PET), and blood analyses for a number of angiogenesis markers. At day 12, none of the patients exhibited a

significant macroscopic clinical response (Fig 2).^{28,37} However, at this early time point, bevacizumab induced a number of assessable effects on tumor vasculature and the cancer cells themselves. Tumor vascular density measurements by immunohistochemistry and blood flow parameters by perfusion CT decreased after bevacizumab infusion.^{28,37} A decrease in blood flow was seen in multiple trials of bevacizumab⁴⁶ and anti-VEGF receptor tyrosine kinase inhibitors.^{35,47,48} Consistent with data from a breast cancer trial, one cycle of bevacizumab treatment alone significantly increased rectal cancer cell apoptosis (Fig 3A).^{37,46} Certain cancer cells (eg, pancreatic, breast) may express functional VEGF receptors, and thus may depend on VEGF for survival.^{46,49} However, the current clinical data showed no increase in overall survival with bevacizumab in these tumor types. Moreover, phase II and III trials have determined that lower doses of bevacizumab have an increased or equivalent efficacy in colorectal and lung cancer.^{50,51} Thus, it remains unclear if the increase in cancer cell apoptosis is the result of direct effects of anti-VEGF therapy on cancer cells, or the result of pruning of vessels in some areas of the tumor.^{36,49} Of particular interest, evaluation in breast and rectal cancer of tumor cell proliferation after bevacizumab treatment showed either no difference⁴⁶ or a clear trend for increased proliferation compared with baseline (Fig 3B).³⁷ These data suggest a normalization of tumor microenvironment by bevacizumab, and may explain the potential synergy between bevacizumab and cytotoxic regimens.

Tumor interstitial pressure measurements were significantly lower after bevacizumab administration (Fig 4). This result is consistent with preclinical^{17,19} and clinical³⁵ data and suggests rapid tumor vascular normalization during anti-VEGF therapy. In contrast, [¹⁸F]FDG uptake in the tumors measured on PET scans remained constant at day 12, but decreased significantly after bevacizumab and chemoradiotherapy treatment (Fig 5).^{28,37,52} Thus, despite the decreased tumor vascular density and blood flow, and increased cancer cell apoptosis, tumor metabolism as assessed by [¹⁸F]FDG activity was unchanged, thus supporting the vascular normalization hypothesis. Additional support of the normalization mechanism has been the observation of increased pericyte coverage in tumor vessels after bevacizumab administration alone.²⁸

In summary, the findings of correlative studies of bevacizumab with chemoradiotherapy in rectal cancer are consistent with an antivascular and vascular normalizing effect of VEGF blockade on the tumor.^{28,37} Tumor responses evaluated by endoscopy, CT, and PET 7 weeks after completion of the combined treatment showed regression with usually only an ulcer remaining in these patients (Fig 2). Complete pathologic responses were seen with this combined regimen, including in a large, invasive, stage T4 carcinoma as determined by ultrasound.⁴⁵

Investigators from Duke University recently reported the results of a phase I trial that evaluated the combination of bevacizumab with capecitabine, oxaliplatin, and radiation therapy in 11 patients with stage II–IV rectal cancer.^{52a} Patients received 50.4 Gy of external-beam radiation therapy to the tumor in 28 fractions. In this study, a dose level of bevacizumab 15 mg/kg on day 1 and then 10 mg/kg on days 8 and 22, oxaliplatin 50/mg² weekly, and capecitabine 625 mg/m² twice each day during radiation days had an acceptable toxicity profile. Two patients had a complete pathological response, and three patients had microscopic disease only. Based on these encouraging results, a phase II study is being pursued. The efficacy signals seen with these regimens remain to be confirmed in ongoing phase II studies, and established in future randomized trials of antiangiogenics with chemoradiotherapy.

In a phase I dose-escalation study in pancreatic cancer at M.D. Anderson Cancer Center (Houston, TX), bevacizumab was administered in one cycle of monotherapy followed by chemoradiotherapy and then again as monotherapy until disease progression.⁴² Patients with inoperable pancreatic adenocarcinoma received bevacizumab 2 weeks before radiotherapy (12

patients each at 2.5, 5.0, 7.5, and 10 mg/kg), every 2 weeks during radiotherapy (50.4 Gy treating the primary tumor and gross adenopathy), and after radiotherapy until disease progression. Capecitabine was administered on days 14 through 52 (650 mg/m² orally twice daily for the first six patients; 825 mg/m² for the remaining patients). Significant acute GI, hand and foot syndrome, and transient hematologic events were uncommon, with protocol-mandated dose reductions of capecitabine for grade 2 toxicity (43% of patients). Among the first 30 patients treated, three patients had tumor-associated bleeding duodenal ulcers, and one had a contained duodenal perforation. No additional bleeding events occurred among the final 18 patients after patients with duodenal involvement by tumor were excluded. Nine (20%) of 46 assessable patients had confirmed partial responses until distant progression for a median of 6.2 months. Four patients underwent pancreaticoduodenectomy without perioperative complication. The median survival was 11.6 months from the start of protocol therapy. In conclusion, concurrent bevacizumab did not significantly increase the acute toxicity of a relatively well-tolerated chemoradiotherapy regimen. However, ulceration and bleeding in the radiation field possibly related to bevacizumab occurred when tumor involved the duodenal mucosa. The encouraging efficacy end points suggest that additional study of bevacizumab with chemoradiotherapy in this highly treatment-refractory cancer is warranted.

FUTURE DIRECTIONS: ESTABLISHING SURROGATE MARKERS FOR ANTIANGIOGENICS WITH CHEMORADIOOTHERAPY

One of the many challenges in the further development of antiangiogenic-chemoradiotherapy combinations is the lack of validated ways of monitoring tumor response to antiangiogenics. Despite recent widespread use of these agents, there are no proven surrogate markers for these therapies.⁵³ These biomarkers are urgently needed to validate the mechanistic hypotheses, identify responsive patients and optimal doses, predict efficacy of regimens that include antiangiogenics, and detect and prevent tumor escape from this type of treatment. These biomarkers would facilitate the optimization of the dose and schedule of bevacizumab for metastatic colorectal, breast, and lung cancer, and extrapolation of the existing efficacy data to multiple other tumor types. In the absence of an overt cytotoxic effect (tumor shrinkage) of the antiangiogenics, other surrogate markers for efficacy must be identified. Significant advances have been made in identifying candidate markers, some of which are newly developed, target-based, and mechanism-based biomarkers (Table 2). Our group is actively pursuing the evaluation of all of these potential biomarkers in multiple trials of antiangiogenics by developing and adapting a variety of techniques.

Where serial biopsies are feasible, tissue analyses for the gene, protein, or cell of interest can be an important platform for both biomarker discovery and evaluation of the targets and of the effects of antiangiogenics.^{28,37,46} In rectal cancer patients, evaluation of serial biopsies before and after treatment showed that bevacizumab decreased the microvascular density in carcinoma tissue by approximately half.^{28,37} The remaining vessels displayed an increased perivascular cell coverage. Similar to some preclinical results, we and others found increases in tumor cell apoptosis and proliferation after bevacizumab treatment alone.^{37,46} Finally, tissue studies may provide critical information regarding cancer and/or tumor stromal cell expression of the molecule targeted by a specific antiangiogenic agent (eg, VEGF, VEGF receptors, or platelet-derived growth factor receptors) or of molecules previously linked to cancer resistance to therapy (eg, survivin).^{35,45}

The ability to measure the interstitial fluid pressure and tissue oxygenation is critical for direct evaluations of vascular function. In many tumor types, these parameters can be evaluated serially and thus provide critical information for an optimized delivery of cytotoxic agents.³⁶

Blood biomarkers are minimally invasive and relatively easy to evaluate in essentially all cancer patients. Our phenotypic studies of blood cells using flow cytometry demonstrated that bevacizumab decreased the number of viable circulating endothelial cells (CECs) and circulating progenitor cells (CPCs) in rectal cancer patients, consistent with its antivasculature effect.^{28,37,60} Whether quantitative evaluation of these cells during treatment has any predictive value for the treatment with antiangiogenics and chemoradiotherapy is currently being investigated in an ongoing phase II trial. In recurrent glioblastoma patients enrolled onto a phase II study, the number of viable CECs predicted tumor response after antiangiogenic treatment with AZD2171 (cediranib [Recentin]; AstraZeneca) monotherapy, whereas CPCs predicted the recurrent growth after drug interruptions.³⁵ We are currently evaluating the kinetics of circulating cells as biomarkers of response in several ongoing or planned trials of antiangiogenics with chemoradiotherapy in glioblastoma patients using a standardized protocol.⁵⁴

Because of the relative ease of performing protein analyses, and benefiting from improved techniques of protein detection, the assessment of changes in angiogenic biomarkers in bodily fluids has been pursued recently in many clinical studies. Most of the results from these studies converge on the conclusion that the sustained but reversible increases in plasma VEGF and placental-derived growth factor and reduction in plasma soluble VEGFR2 are valid pharmacokinetic/pharmacodynamic markers that the VEGF pathway has been blocked.^{35, 37,55–57,61} Whether the kinetics of these factors can predict tumor response to antiangiogenic or combined treatments is less clear. Another group reported in a phase II/III study that lower baseline plasma soluble intracellular adhesion molecule-1 levels, but not VEGF or basic fibroblast growth factor, predicted overall survival after bevacizumab with chemotherapy in metastatic lung cancer patients.⁶² Of great interest for the field would be to identify biomarkers that predict disease progression through therapy with antiangiogenics and chemoradiotherapy. Our data from a recently completed phase II study of AZD2171 in recurrent glioblastoma patients showed a significant correlation between basic fibroblast growth factor and stromal cell–derived factor-1 alpha measured at multiple time points and tumor progression, as well as between these two cytokines and tumor vascular changes (ie, increased tumor vessel size) measured by magnetic resonance imaging during treatment.³⁵

These preliminary data on blood biomarkers warrant additional studies of these circulating cells and cytokines in trials of different antiangiogenics with chemoradiotherapy in patients with different pathologies. With the development and improved flow cytometry and protein array analysis techniques and subsequent standardization, circulating cell and plasma protein measurements hold great promise for identification of valid biomarkers for antiangiogenics alone or with chemoradiotherapy. In addition, this may help clarify any role that blood CECs, CPCs, or cytokines play in tumor response or escape from anti-VEGF therapy.

Finally, protein measurements in urine have become increasingly feasible,^{63–65} and exploring them during therapy might offer independent surrogate markers for the effect of antiangiogenics.

In addition to their use to assess responses, noninvasive techniques can measure functional parameters and may offer surrogate markers for therapy for tumor type or location. We and others have evaluated tumor response to antiangiogenics in the context of clinical trials, using techniques that include dynamic magnetic resonance imaging, CT, and PET.^{2,28,37,39,40, 45–47} These imaging techniques are improving constantly and are being pursued in clinical trials by our own team and others. However, the cost of such investigations is an important hurdle for larger trials or routine evaluations.

In conclusion, demonstration of the clinical utility of angiogenics when combined with chemotherapy and radiation awaits the completion of future phase III randomized clinical trials. Nevertheless, promising responses and important lessons have emerged from early phase I and II trials. Mechanistic insights and candidate biomarkers for antiangiogenic therapy have been reported. These can be explored further in both preclinical and clinical settings. On the basis of all of this knowledge gained from bench-to-bedside-and-back approaches, the clinical application of combined antiangiogenics and chemoradiotherapy has started to seem within reach.

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Fig 1.

Antitumor effects of antiangiogenics. In normal tissue, there is balance between (A) proangiogenic (pro) and antiangiogenic (anti) factors; in (B) tumors, proangiogenic signals preponderate and induce an abnormal vasculature. Antiangiogenics are intended to kill angiogenic vessels and starve tumors, but most tumors relapse, presumably (D) using pathways not blocked by the antiangiogenic agent used; alternatively, antiangiogenesis agents transiently normalize the vasculature, (C) making it more efficient for drug and oxygen delivery. Adapted with permission.^{21,22}

Fig 2.

Rectal tumor response to bevacizumab (BV) and chemoradiotherapy. Sigmoidoscopy at day 12 showed no response in any patient after bevacizumab alone (A, B); a marked tumor response was seen presurgery in all patients, with an ulcer and no evidence of macroscopic disease. Histopathological evaluation of surgical specimens usually showed marked tumor regression. H&E, hematoxylin and eosin stain. Adapted with permission.^{28,37}

Fig 3.

Effect of bevacizumab (BV) on cancer cells. The percentage of tumor cell undergoing (A) apoptosis and (B) proliferation was determined in serial biopsies by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling and immunostaining for proliferation cell-nuclear antigen, respectively. Cell apoptosis increased in seven of eight patients and cell proliferation increased in four of eight patients. Adapted with permission.³⁷

Fig 4. Effect of bevacizumab on interstitial fluid pressure (IFP). (A) Tumor IFP measurement at sigmoidoscopy using the wick-in-needle technique. At day 12 after the first bevacizumab dose, tumor IFP decreased in seven of nine patients analyzed, who had higher IFP values before treatment. Tx, treatment. Adapted with permission.^{28,37}

Fig 5. Rectal tumor response to bevacizumab and chemoradiotherapy evaluated by positron emission tomography. [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) uptake was evaluated before treatment (Tx), on day 12, and presurgery (tumor highlighted in box). No change in FDG uptake was observed on day 12 after bevacizumab infusion. Nevertheless, the FDG uptake was significantly lower at presurgery compared with pretreatment (* $P < .01$). Adapted with permission.^{28,37,52}

Table 1**Differences Between Normal Vasculature and Tumor Vasculature**

Normal Vasculature	Tumor Vasculature
Organized	Disorganized
Evenly distributed	Unevenly distributed
Uniformly shaped	Twisted
Nonpermeable	Leaky
Vascular pressure is greater than interstitial pressure	Vascular pressure is similar to tumor interstitial pressure
Properly matured	Immature
Supporting cells present (eg, pericytes)	Supporting cells absent
Appropriate membrane protein expression	Inappropriate membrane protein expression
Independent of cell survival factors	Dependent on cell survival factors (eg, VEGF)
Homogenous oxygenation of tissue within O ₂ diffusion limit	Focal hypoxic and anoxic regions

Abbreviation: VEGF, vascular endothelial growth factor.

Table 2**Potential Surrogate Markers for Antiangiogenics**

Marker	Type of Evaluation	Current data	Comments/Limitation	Reference
Tissue biopsy	Protein analyses of tumor tissue; genomic analyses of tumor tissue	Antiangiogenics can decrease MVD and increase perivascular cell coverage. Antiangiogenics can increase cancer cell apoptosis and proliferation. K-RAS, BRAF, or P53 mutation status; P53, VEGF, or TSP2 expression; or microvascular density at baseline do not predict efficacy of bevacizumab.	Invasive, not easily available in many tumors	Willett et al. ^{28,37} Wedam et al. ⁴⁶ Jubb et al. ⁵³ Ince et al. ⁵⁸
IFP	Tumor tissue IFP	Bevacizumab decreases IFP in rectal cancer patients	Difficult accessibility in some tumors	Willett et al. ^{28,37}
Tissue oxygenation	Tumor interstitial oxygen tension	NA	Lack of accessibility in some tumors	Dunst et al. ⁵⁹
Blood CECs	Concentration of viable CECs	Bevacizumab decreases viable CECs in rectal cancer patients. Viable CECs correlate with glioblastoma recurrent growth on AZD2171 treatment.	Minimally invasive; standardized methods and protocols available	Willett et al. ^{28,37} Batchelor et al. ³⁵ Duda et al. ⁵⁴
Blood CPCs	Concentration of CPCs	CPCs correlate with glioblastoma relapse after AZD2171 treatment		Batchelor et al. ³⁵
Protein level in bodily fluids	Protein concentration in plasma, serum, urine, ascites, pleural effusions	VEGF, PlGF, sVEGFR2, sICAM1, bFGF, SDF1 α may correlate with treatment and/or tumor response to antiangiogenics	Minimally invasive or noninvasive; standardized and high-throughput methods available	Batchelor et al. ³⁵ Willett et al. ³⁷ Dreys et al. ^{55,57} Motzer et al. ⁵⁶
CT imaging	Blood flow and volume, permeability–surface area product mean transit time	Blood flow and volume decreased by bevacizumab in rectal cancer	Noninvasive, relatively low resolution, measurement of composite parameters	Willett et al. ^{28,37} Liu et al. ⁴⁷
PET imaging	Tracer uptake, tissue oxygenation	[¹⁸ F]FDG uptake is unchanged by bevacizumab alone, but significantly decreased by bevacizumab with chemoradiation	Noninvasive, relatively low resolution, measurement of composite parameters	Willett et al. ^{28,37}
MRI	Blood flow, permeability	Bevacizumab, PTK787, AZD2171, and AG-013736 decrease blood flow and permeability. AZD2171 also decreases relative	Noninvasive, relatively low resolution, measurement of composite parameters	Batchelor et al. ³⁵ Pope et al. ³⁹ Mross et al. ⁴⁸

Marker	Type of Evaluation	Current data	Comments/Limitation	Reference
		vessel size, and both bevacizumab and AZD2171 reduce peritumor edema.		

Abbreviations: MVD, microvascular density; BRAF, B-Raf proto-oncogene serine/threonine-protein kinase; VEGF, vascular endothelial growth factor; TSP2, thrombospondin-2; IFP, interstitial fluid pressure; NA, not applicable; CEC, circulating endothelial cells; CPC, circulating progenitor cells; PlGF, placental-derived growth factor; sVEGFR2, soluble vascular endothelial growth factor receptor 2; sICAM1, soluble intracellular adhesion molecule 1; bFGF, basic fibroblast growth factor; SDF1 α , stromal cell-derived factor alpha; CT, computed tomography; PET, positron emission tomography; [^{18}F] FDG, [^{18}F]fluorodeoxyglucose; MRI, magnetic resonance imaging.