Antiangiogenesis Treatment for Glioblastoma Multiforme: Challenges and Opportunities

Eric T. Wong, MD, and Steven Brem, MD, Boston, Massachusetts, and Tampa, Florida

Key Words
Glioblastomas, angiogenesis, vascular neuroimaging, treatment

Abstract
Angiogenesis is a major hallmark of cancer cells, and glioblastomas are among the most angiogenic tumors. The cascade of angiogenesis is probably initiated by hypoxia, leading to the production of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Both VEGF and bFGF have paracrine effects on endothelial cells, pericytes, or both, causing the formation of hyperpermeable tumor blood vessels. Advanced MRI techniques, such as dynamic contrast-enhanced, dynamic susceptibility, and arterial spin labeling MRI, have provided semiquantitative measurements of tumor vascular permeability and perfusion. A decrease in vascular permeability and perfusion can be detected after antiangiogenesis drug treatment, either with monoclonal antibody such as bevacizumab that sequesters VEGF, or small-molecule VEGF receptor tyrosine kinase inhibitors. Therefore, antiangiogenesis therapies are being increasingly adopted for treating glioblastomas. However, caution must be exercised because neural stem cells are also sensitive to antiangiogenesis drugs and the combined effect of ionizing radiation. This article summarizes 30 years of laboratory and clinical research on glioblastoma angiogenesis and discusses its underlying biology, clinical trial results, vascular neuroimaging, and the potential side effects of antiangiogenesis treatment. (JNCCN 2008;6:515–522)

Glioblastoma multiforme is a highly angiogenic malignancy. In fact, initial studies on tumor angiogenesis were performed on specimens obtained from patients with glioblastomas. However, it took more than 30 years to move from the conception of antiangiogenesis therapy in the laboratory to clinical therapies approved by the FDA. This realization was fueled by advances in knowledge of the underlying biology of tumor angiogenesis, identification of relevant targets of angiogenesis, and advances in neuroimaging that enabled clinicians to see the effect of antiangiogenic drugs on tumor vasculatures. This article discusses issues relevant to antiangiogenesis treatments of glioblastomas, ranging from the biology of glioblastoma angiogenesis to the clinical evaluation of antiangiogenesis treatment efficacy.

The Biology of Tumor Angiogenesis
The essential trigger for tumor angiogenesis is hypoxia (Figure 1). Within hours of exposure to hypoxic conditions, C6 glioma cells upregulate the expression of vascular endothelial growth factor (VEGF) messenger RNA and other proangiogenic and oncogenic peptides. The expression of VEGF is tightly regulated by hypoxia-inducible transcription factor (HIF) complex, with the functions of HIF-1α and HIF-2α best characterized. Under normoxic conditions, both HIF-1α and -2α are rapidly polyubiquinated through interaction with von Hippel-Lindau tumor suppressor complex, leading to their degradation by proteosomes. However, under hypoxic conditions, HIF-1α and -2α are stabilized and transcription of angiogenic factors ensues. Therefore, it is not surprising that germline mutations or deletions of von Hippel-Lindau tumor suppressor gene led to the formation of multiple types of vascularized tumors, including hemangioblastomas, renal cell carcinoma, retinal angiomas, pheochromocytomas, and tumors of the endolymphatic sac. A paracrine mechanism exists between glioblastoma and endothelial cells (Figure 1). Highly proliferative glioblastoma cells situated next to areas of necrosis secrete
Whether this thinking is correct remains to be determined, and it is likely that multiple growth factors are involved in the growth of glioblastomas (Figure 1). bFGF has an autocrine proliferative effect because glioblastoma cells have an elevated expression of FGF receptor 1. More importantly, both VEGF and bFGF probably act in concert in the recruitment of pericytes. VEGF enhances the secretion of platelet-derived growth factor-BB (PDGF-BB) by endothelial cells, and FGF-2 enhances the expression of PDGF receptor β on pericytes. These pericytes then solidify immature vasculatures, making them less permeable.

**Anti-Angiogenesis Targets in Glioblastomas**

The pharmacologic blockade of tumor angiogenesis has multiple targets. Drugs can be designed to sequester circulating VEGF, block the function of VEGFR tyrosine kinases or their downstream effector protein kinases, or interfere with the migration and proliferation of endothelial cells and pericytes.

Bevacizumab, a humanized monoclonal antibody, is the prototypic drug that sequesters circulating VEGF and causes regression of immature tumor vasculatures. One effect could be vascular normalization, as supported by decreasing gadolinium enhancement and edema on MRI. When bevacizumab is combined with cytotoxic chemotherapy, such as irinotecan, it can produce an objective response rate (complete + partial response) of 57% and a 6-month progression-free survival of 46% (Figure 2). The combination is substantially better than the 6% objective response rate and 15% 6-month progression-free survival from salvage cytotoxic chemotherapy alone. Whether this dramatic effect is a result of synergism from combining antiangiogenesis drug and cytotoxic chemotherapy is under study in a European randomized phase II study of bevacizumab alone versus bevacizumab plus irinotecan. Notably, high VEGF level in archival malignant gliomas correlated with radiographic response to bevacizumab but not patient survival. Furthermore, patients with tumors having high carbonic anhydrase-9 (CA9) and HIF-2α had the worst survival compared with those having low CA9, HIF-2α, or both, suggesting that the level of tissue hypoxia is related to the aggressiveness of malignant gliomas.

Another possible target of bevacizumab is the perivascular niche in which glioblastoma stem cells reside. Glioblastoma stem cells probably require trophic factors, such as brain-derived neurotrophin,
Antiangiogenesis Treatment for Glioblastoma Multiforme

VEGF-C, and pigment epithelium-derived growth factor, from endothelial cells in the perivascular niche. Bevacizumab may disrupt these endothelial cells and block the supply of these trophic factors. Conflicting reports also suggest that glioblastoma stem cells have an angiogenesis-independent but highly invasive phenotype, suggesting that blocking angiogenesis alone in glioblastoma is not enough.

Blocking VEGFR tyrosine kinase activity could also lead to antiangiogenesis. This was shown experimentally with DC101, a monoclonal antibody against VEGFR 2, in mice with implanted U87 glioblastomas into their brains. As hyperpermeable vasculatures were pruned away by DC101, increased tissue oxygenation acted synergistically with external beam radiation in controlling U87 glioblastomas. Interestingly, this “normalization window” peaked at day 5 of DC101 treatment. A similar effect on vascular normalization and reversal of vascular hyperpermeability was seen when AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, was used to treat patients with recurrent glioblastomas.

When metronomic temozolomide, a drug with proven efficacy against glioblastomas, was used to treat mice implanted with C6 gliomas, evidence of antiangiogenesis was seen. Although tumor vasculatures have increased permeability and blood flow (or perfusion), quantifying changes induced by antiangiogenic drugs in these parameters is problematic. For instance, when a bolus of gadolinium travels to the blood vessels in a tumor, some leaks into the interstitial space while the rest remains in the blood vessel as part of the bulk flow.

Neuroimaging of Antiangiogenesis Drug Efficacy

![Figure 2](https://example.com/figure2.png)

Figure 2 Response after bevacizumab and irinotecan in recurrent glioblastoma multiforme in left parietal brain. (A) Before treatment, significant edema and midline shift were detected in the fluid attenuated inversion recovery (FLAIR) sequence and on significant gadolinium enhancement. The arterial spin labeling sequence (ASL) showed hyperfusion (arrow) and white matter tracts were absent in the edematous brain on diffusion tensor imaging (DTI; arrows). (B) After 1 cycle of bevacizumab and irinotecan, a significant decrease in edema was seen on FLAIR and in gadolinium enhancement (Post-Gad). The ASL showed normalized perfusion (arrow), and white matter tracts reappeared on DTI (arrows).
Therefore, changes in permeability can alter perfusion and vice versa (Figure 3).44 Nevertheless, several semiquantitative MRI techniques have been developed, such as dynamic contrast-enhanced (DCE), DSC, and arterial spin labeling (ASL) MRI, to monitor glioblastoma angiogenesis. Knowing the strengths and limitations of these techniques aids proper evaluation of antiangiogenic drugs.

In DCE MRI, the amount of gadolinium uptake by the tumor is measured over time, and the velocity of gadolinium uptake provides a semiquantitative measure of perfusion. In a study using DCE MRI, perfusion as measured by peak gadolinium uptake velocity in T1 correlated with survival in anaplastic gliomas but not in glioblastomas.49 This lack of survival correlation is probably caused by glioblastoma vasculatures that are maximally hyperperfused, whereas the vasculatures in anaplastic gliomas are less hyperperfused and have more variability in perfusion. As a result, patients with highly perfused anaplastic gliomas have shortened survival compared with those who have low perfusion.

DSC MRI can also estimate vascular permeability, expressed as a transendothelial transfer constant $K_p$. $K_p$ has been shown to correlate with histologic grade in gliomas and can change with antiangiogenesis treatments.46 For example, $K_p$ dropped in breast carcinomas when patients were treated with AG-013736, a pan-VEGF, PDGF, and c-Kit receptor tyrosine kinase inhibitor.47

In DSC MRI, perfusion is measured by intravascular susceptibility changes induced by gadolinium in T2, or $T_2^*$ effect, theoretically providing a better measure of capillary perfusion than DCE MRI.44 However, in both DCE and DSC MRI, the permeability component, or $K_p$ and $K_{trans}$, respectively, confounds perfusion measurement in tumors because of the underlying assumption that gadolinium is nonpermeable. This is represented mathematically by 2 unsolved variables in the perfusion equation, cerebral blood flow, and $C(t)_{tissue}$ (Figure 3). In reality, this assumption is untrue, because glioblastomas and anaplastic gliomas have leaky vasculatures.

A second problem associated with DCE and DSC MRI is the phenomenon of temporal dispersion, which would underestimate perfusion because the convolution function assumes the same durations of contrast-induced changes in the vasculature as for $C(t)_{artery}$ and $R(t)$ (Figure 3). Nevertheless, the permeability constant, $K_{trans}$, from DSC MRI has been shown to decrease in patients with glioblastomas after AZD2171 treatment.55

ASL MRI may provide a more accurate measurement of tumor perfusion than DCE and DSC MRI because it uses water as the labeled agent. Unlike gadolinium, water freely permeates across the intravascular compartment into interstitial space and is at equilibrium, without a concentration gradient where gadolinium is used, between these 2 compartments. ASL MRI uses magnetic fields to alter the signal from inflowing arterial blood, causing a small change in signal intensity within tissue, which can be related to blood flow.48 ASL has been widely used in animal studies and humans. Versions of the technique have been validated in animals with microspheres49 and in the normal human brain with $H_2O$50 PET.51 Initial clinical explorations of the technique have been performed in cerebrovascular disease,51–53 epilepsy,54 and dementia.52 Recent reports of echoplanar ASL techniques applied to brain tumors have shown increased perfusion in pretreatment glioma55 and correlated response of brain metastases to radiation therapy and ASL perfusion.56

Advances in MRI techniques have made high-quality, robust ASL neuroimaging possible. Initial implementations of ASL offered limited slice coverage, used susceptibility-sensitive echoplanar imaging, sometimes showed motion artifacts, and often used insufficient delays between labeling and imaging, causing unreliable results. More recent implementations have
Antiangiogenesis Treatment for Glioblastoma Multiforme

stressed the use of robust fast spin echo or hybrid imaging techniques, background suppression to reduce motion artifacts, whole brain 3-dimensional acquisition, and longer delays suitable for patients. Three-dimensional acquisition also permits reslicing to arbitrary planes for comparison with anatomic, contrast-enhanced MRI (Figure 4). Data acquisition using a high-field-strength 3.0- or 4.0-T magnet also improves the signal-to-noise ratio up to 50% (Figure 2 and 4B). Therefore, these improvements can make ASL acquisition a routine part of vascular neuroimaging in patients with glioblastoma.

Limitations of Antiangiogenesis Therapies and Future Directions

Antiangiogenesis treatments have several limitations. First, the current response criteria, or Macdonald’s criteria, may be insufficient to accurately assess glioblastoma response to antiangiogenic drugs, because tumor size is estimated based on gadolinium leakage from hyperpermeable vasculatures. When antiangiogenic drugs decrease vascular permeability, they may alter the image of glioblastomas on gadolinium-enhanced MRI, without impacting the underlying tumor mass. Therefore, adjunctive measures of tumor response,

Figure 4 Corresponding post–gadolinium-enhanced T1-weighted (A) and arterial spin labeling sequence (ASL; B) MRI images in a patient undergoing treatment for recurrent glioblastoma. Serial post–gadolinium-enhanced T1-weighted images did not detect tumor recurrence (arrows in A) until week 30 in the inferior and posterior margins of the resection cavity. However, hyperperfusion was detected by ASL at week 24, 6 weeks earlier than gadolinium enhancement (arrows in B), suggesting that hyperperfusion is a more sensitive parameter for detecting glioblastoma recurrence than hyperpermeability changes.
such as $^{18}$F-fluorothymidine (FLT) or $^{11}$C-methylmethionine PET, would be necessary.\textsuperscript{62,63}

Unlike $^{18}$F-fluorodeoxyglucose PET, FLT-PET and $^{11}$C-methylmethionine PET have higher signal-to-noise ratio and their usefulness is not impaired by the high rate of cerebral glucose use.\textsuperscript{62,64} In a clinical trial using bevacizumab plus irinotecan for recurrent glioblastomas, FLT-PET correlated with MRI response and patient survival. Patients who experienced an FLT-PET metabolic response lived 3 times longer than those who did not.\textsuperscript{65} However, additional research is needed to evaluate the positive and negative predictive values of FLT-PET and $^{11}$C-methylmethionine PET before they are incorporated into routine clinical practice.

Notably, not all patients experience response to anti-VEGF or AZD2171 treatment. Among 20 patients who experienced response in the bevacizumab and irinotecan trial,\textsuperscript{66} only 1 complete response was seen; the remainder were partial responses. Similarly, a spectrum of responses were seen in patients who received AZD2171.\textsuperscript{67} This variability may be caused by either a feedback increase of VEGFR expression in endothelial cells when blocking VEGF alone, or elevated VEGF expression from tumor cells to overcome VEGFR blockade. Therefore, simultaneous VEGF and VEGFR blockade may offer a better therapeutic response than blocking either ligand or receptor alone.

Antiangiogenesis therapy may promote infiltrative tumor growth that lack gadolinium enhancement on MRI. In a retrospective quantitative volumetric analysis of recurrence pattern in malignant gliomas treated with bevacizumab and various cytotoxic chemotherapies, Norden et al.\textsuperscript{67} noted that patients who experienced response had a 7.1-fold increase in the volume of fluid-attenuated inversion recovery (FLAIR) hyperintensity to the volume of gadolinium enhancement, whereas those who did not experience response had only a 2.4-fold increase. However, infiltrative tumors in the FLAIR-hyperintense regions have not been confirmed by pathology, FLT-PET, or $^{11}$C-methylmethionine PET.

Bleeding and thrombosis are potentially deadly complications of antiangiogenesis treatments for glioblastomas. Patients with glioblastomas already have an increased risk for thromboembolism, estimated at 30%.\textsuperscript{68,69} Two clinical trials using bevacizumab plus irinotecan\textsuperscript{21,65} reported no spontaneous hemorrhage, and only 4 patients (11%) in 1 of the trials developed thromboembolism requiring treatment discontinuation.\textsuperscript{21} In the AZD2171 trial, no bleeding or thrombosis complication was reported, but the follow-up time was short.\textsuperscript{67} Whether anticoagulation in this setting would increase the risk of hemorrhage into glioblastomas is still unknown.

Rebound cerebral edema is a possibility after withdrawal of antiangiogenesis treatment. The AZD2171 trial showed that vascular normalization is reversible, and that tumor enhancement volume and permeability increased substantially during a 14-day drug holiday.\textsuperscript{65} The reversibility of vascular enhancement once antiangiogenesis drugs are discontinued suggests that rebound cerebral edema may occur and necessitate the use of prophylactic dexamethasone.

Lastly, bevacizumab may induce a reversible posterior leukoencephalopathy syndrome, characterized by vasogenic edema in the occipital brain.\textsuperscript{70,71} This unusual syndrome is believed to be caused by a drop in circulating VEGF leading to endothelial dysfunction.\textsuperscript{70,72} However, why only certain individuals develop this syndrome and why it occurs exclusively in the posterior cerebral circulation is still unclear.

Another potential complication of antiangiogenesis therapy is the toxicity imposed on neural stem cells. These cells reside in the subventricular zone,\textsuperscript{72,73} and their self-renewal and neuronal differentiation require a supply of trophic factors, such as VEGF and brain-derived neurotrophin, from endothelial cells in the perivascular niche.\textsuperscript{74} If this supply of trophic factors is disrupted by antiangiogenesis treatment, either through sequestration of VEGF by bevacizumab or blockade of VEGFR tyrosine kinases on endothelial cells, patients may experience delayed cognitive problems.

The addition of bevacizumab to radiation may intensify delayed cognitive problems. Bevacizumab is being added to involved-field cranial irradiation plus temozolomide in patients with newly diagnosed glioblastomas.\textsuperscript{75} Ionizing radiation alone can cause apoptosis of neural stem cells and impair neurogenesis and differentiation.\textsuperscript{76,77} The addition of antiangiogenic drugs, which can disrupt the normal function of perivascular niche, may potentiate the toxicity of ionizing radiation to neural stem cells, causing delayed cognitive deficits. This may be consistent with the dictum that any treatment for glioblastomas could have off-target side effects. The challenge is to design treatments that can maximize efficacy while minimizing toxicities.
Acknowledgements
The authors would like to thank David Alsop, PhD, for his input on the vascular MRI techniques, and Nadine Linendoll, PhD, MDiv, APRN, for her helpful comments.

References


