Antiangiogenic Therapy for Glioblastoma: New Directions

Edited by Tom Mikkelsen, M.D., FRCP(C) and David A. Reardon, M.D.

Glioblastoma multiforme (GBM), a highly aggressive malignant primary brain tumor, is among the most vascularized of all solid tumors, and relies upon angiogenesis for growth and histological progression\(^1,2\). Angiogenesis in GBM, like all solid tumors, is mediated primarily by vascular endothelial growth factor (VEGF), an endogenous cytokine that stimulates capillary sprouting from pre-existing vessels toward VEGF-expressing tumor cells. Tumor VEGF expression and angiogenesis are mainly hypoxia-driven, but can also be promoted by other vascular cytokines and constitutively expressed as a result of genetic tumor mutations\(^1\). A number of other proangiogenic factors also promote angiogenesis in GBM, including basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), angiopoietin-2 (Ang-2), stem cell factor, and hepatocyte growth factor/scatter factor through different signaling pathways\(^3,4\).

VEGF is also the main vascular permeability factor, and is therefore the primary culprit behind vascular cerebral edema, a major cause of morbidity and mortality in brain tumor patients. Overexpression of VEGF by GBM tumor cells degrades the capillary basement membranes of tumor vessels, causing them to become hyperpermeable and leak plasma fluid and proteins from the intravascular compartment into the brain parenchyma\(^3\). This process causes extensive vasogenic edema, increased interstitial fluid pressure, and mass effect characteristic of advanced GBM\(^2,3\). A major cause of death in GBM patients is cerebral herniation, which results primarily from cerebral edema and intracranial hypertension\(^3\). Corticosteroids are used to temporarily alleviate brain edema and reduce mass effect, but are associated with a number of serious dose-limiting adverse effects.

From the Editor-in-Chief

Malignant gliomas remain one of the most challenging tumors to treat. In 2009, the FDA granted accelerated approval to bevacizumab for recurrent glioblastoma multiforme (GBM), making it the first clinically validated antiangiogenic therapy for this tumor type. With bevacizumab now in front-line registration trials for GBM, and a number of other targeted therapies in advanced clinical trials, antiangiogenic treatments are poised to change the treatment landscape for this devastating disease. In this issue of Targeting Tumor Angiogenesis, Tom Mikkelsen, M.D., FRCP(C), Co-Director of the Hermelin Brain Tumor Center, Henry Ford Hospital, and David Reardon, M.D., Associate Deputy Director of The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, provide their perspective on new clinical data on antiangiogenic treatments in GBM, and how researchers are using innovative imaging techniques to measure response to antiangiogenic therapy.

— William W. Li, M.D., President, The Angiogenesis Foundation

Antiangiogenic Agents for Glioblastoma

Until recently, few treatment options were available for recurrent GBM. Single-agent irinotecan (CPT-11), a topoisomerase I inhibitor used in the relapsed setting, produces response rates of ≤15\(^\%\). Six-month progression-free survival (PFS6) rates in relapsed GBM with single-agent irinotecan typically ranges from 9-21\(^\%\), and median overall survival (OS) is ≤30 weeks\(^4\). Antiangiogenic therapies, which include the anti-VEGF monoclonal antibody bevacizumab (Avastin\(^\circledast\); BV) and a number of orally administered small molecule agents, are producing dramatic radiological responses in some GBM patients (Figure 1) and prolonging PFS relative to historical controls in the relapsed setting (see table). Newer, sophisticated magnetic resonance imaging (MRI) techniques now being utilized in conjunction with anti-VEGF therapy show that these agents transiently ‘normalize’ tumor vasculature and restore integrity of the blood brain barrier, often with important clinical implications\(^2\). Alleviation of vasogenic edema is associated with relief of neurological symptoms, reduction of mass effect, and a reduced need for steroids in many GBM patients\(^3,5\).

Bevacizumab

In May 2009, BV became the first clinically validated antiangiogenic therapy for GBM after the FDA granted it accelerated approval in the relapsed setting. In the initial phase 2 study conducted in 35 patients with recurrent GBM, BV in combination with irinotecan produced a radiographic response rate of 57\(^\%\)—dramatically higher than the 5\(^\%\) response rate typically seen with temozolomide (TMZ) therapy at first recurrence\(^5\). This was followed by a second phase 2 study of single-agent BV in 48 heavily pretreated GBM patients\(^6\). The PFS6 was 29\(^\%\), and the overall survival (OS) rate at 6 months was 57\(^\%\). In addition, alleviation of cerebral edema was observed in 24 patients (50\%), and 15 of 26 (58\%) patients receiving corticosteroids for edema were able to decrease their corticosteroid dose by an average of 59\%.

The clinical efficacy of BV for recurrent GBM was confirmed in a larger, randomized, non-comparative phase 2 study (BRAIN) that included 167 patients who received BV (10 mg/kg) with or without irinotecan\(^4\). The reported PFS6 rate was 43\% for single-agent BV and 50\% for BV plus irinotecan. Objective response rates were 28.0\% and 38.0\%, and median OS times were 9.2 mo. and 8.7 mo. for single-agent BV and combination therapy, respectively. There was also a trend for a decreased reliance on corticosteroids and reduced steroid doses during treatment with BV. The most common adverse events associated with single-agent BV were fatigue (45\%), headache (37\%), and hypertension (30\%), while in the BV/irinotecan combination group they were fatigue (76\%), diarrhea (75\%), and nausea 67\%).
Bevacizumab in recurrent GBM: Results from three phase 2 studies

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BV has also demonstrated promising activity in the front-line GBM treatment setting. In a phase 2 study, 75 patients with newly diagnosed GBM were treated with standard radiotherapy (RT) with adjuvant TMZ plus BV (10 mg/kg)2. Upon completion of RT, patients received 6 cycles of BV plus TMZ and irinotecan. At a median follow up of 9 months, 81% of patients were alive and progression free. Although treatment and follow-up on this study is ongoing, 22 patients completed the full 6 cycles of BV plus TMZ/irinotecan, 17 of whom had a cold PET scan during treatment. In a second phase 2 study conducted in 70 newly diagnosed patients treated with TMZ/RT plus BV (10 mg/kg), researchers reported a PFS6 rate of 89% and a 12-month PFS rate of 58%8. By point of comparison, the respective 6- and 12-month PFS rates with front-line RT/TMZ from a landmark, randomized phase 3 trial were 54% and 27%9. Based on the these promising phase 2 data, two randomized, placebo controlled phase 3 trials of RT/TMZ plus BV have been initiated in newly diagnosed GBM, with the primary endpoints of PFS and OS.

An important question in GBM treatment is how to proceed with patients who experience disease progression while on anti-VEGF therapy. For patients on BV, there appears to be scant evidence thus far to support continuing this agent through progression, or for adding irinotecan to BV in the setting of progressive disease6. The previously described phase 2 study of single-agent BV in 48 relapsed GBM patients included a post-progression phase of 19 patients treated with BV plus irinotecan following progression on single-agent BV6. Twelve (71%) patients experienced tumor progression after just one cycle of irinotecan plus BV, and 18 patients (95%) had progression by the second cycle. Only 1 patient had a partial response (PR) based on Levin response criteria, and the median time-to-progression (TTP) was just 30 days. More recently, a small retrospective study evaluated 24 recurrent GBM patients who received a BV-containing salvage regimen following progression on an anti-VEGF tyrosine kinase inhibitor (sorafenib, cediranib, pazopanib, or sunitinib) received as part of a phase 1 or phase 2 clinical study10. Although partial radiographic responses were reported in 6 of 24 patients (21%) who received BV-containing salvage therapy, there was little evidence of sustained clinical benefit, with a median TTP of 8 weeks and PFS6 of 12.5%.

Preclinical research has shown that anti-VEGF therapy, while initially efficacious, promotes the compensatory expression of multiple proangiogenic and proinvasive factors in GBM11. These alternate factors likely contribute to a rebound in tumor angiogenesis following initial suppression, and possibly to a diffuse and invasive pattern of tumor recurrence sometimes observed in GBM patients who have received anti-VEGF therapy12. A major challenge going forward will be to validate new therapies and combinations to thwart anti-VEGF tumor escape in GBM, perhaps through the concurrent use of agents that simultaneously target different proangiogenic and proinvasive signaling pathways11. In this vein, there are multiple ongoing studies combining anti-VEGF therapy with newer chemotherapy agents and drugs that target other signaling pathways involved in GBM tumor cell proliferation, and their results are eagerly awaited.

**Other novel antiangiogenic agents in development for GBM**

In addition to BV, a large number of orally administered novel agents that target intracellular receptors involved in angiogenesis, as well as tumor cell proliferation, migration, and survival, are under investigation. One of these, cediranib (AZD2171; Recentric™), a small molecule oral tyrosine kinase inhibitor (TKI) of VEGF receptor-2, PDGF receptors- and -β, and stem cell growth factor-receptor (c-KIT), was evaluated as a single agent in a phase 2 trial in 30 patients with recurrent GBM13. Sixteen of 30 patients (56%) had a radiographic response, the PFS6 was 26%, and the median PFS and OS were 17 and 32 weeks, respectively. Moreover, 8 of 11 patients who required steroids during treatment had their dose reduced, and 3 patients discontinued steroids altogether. Two ongoing trials should provide important data on cediranib as part of combination therapy; one is a randomized phase 1b/3 trial of cediranib in combination with lomustine (CCNU) in recurrent GBM, and the second, a phase 1b/2 study of TMZ/RT plus cediranib in newly diagnosed GBM.

Another promising antiangiogenic agent under investigation for GBM is cilengitide (EMD121974), a selective inhibitor of the αvβ3, αvβ5 integrins. Integrins are transmembrane receptors expressed by both GBM cells and tumor vasculature that, when activated by extracellular ligands, facilitate tumor cell migration, proliferation, survival, and angiogenesis14. Cilengitide was evaluated in three recent phase 2 studies in both recurrent and front-line GBM treatment settings. In the first study in recurrent GBM, 81 patients received single-agent cilengitide twice weekly at doses of either 500 mg (n = 41) or 2000 mg (n = 40)14. Anti-tumor activity favored the 2000 mg dosing arm, with a PFS6 of 15%, a response rate of 13%, and median OS of 9.9 mo. Among the 7 patients (9%) who achieved a PR, the median PFS was 17 months (range, 10.8 mo. to > 36 mo.).

Results from two phase 2 studies in newly diagnosed GBM, 010 and NABTT 0306, were recently reported. In the 010 study, 52 newly
Targeting Cells and Pathways in Glioblastoma

During tumor angiogenesis, endothelial cells recruit pericytes to stabilize blood vessels perfusing tumors. Endothelial cells also provide paracrine factors to tumor cells which, in turn, release growth factors that sustain angiogenesis. Glioma stem cells also associate directly with endothelial cells in the perivascular stem cell niche, a source of new tumor cells.

**PDGF-B**

**PDGFR-β**

**PI3K**

**Akt**

**eNOS**

**Caspase-9**

**CELL ACTIVATION**

**VESSEL MATURATION**

**Ras**

**Raf**

**MEK**

**ERKp38MAPK**

**Flt-3**

**RET**

**Additional Growth Factors**

**c-Kit**

**p38MAPK**

**Ras**

**Raf**

**MEK**

**ERK**

**Src**

**Rac1**

**JNK**

**PI3K**

**Akt**

**mTOR**

**CELL CYCLE**

**Rb**

**CDK**

**cyclin**

**p21**

**p53**

**EGFR MET**

**2**

**Targeted Agents**

(Targets shown in diagram above and to the right)

- Bevacizumab (Avastin®)
- Cediranib (AZD2171, Recentin®)
- XL184
- Proliferation
- Migration
- Angiogenesis
- Vascular permeability
- Lymphangiogenesis

**EDENDHELIAL CELL**

The vascular endothelial growth factor (VEGF) family (A-D) binds to receptors VEGFR-1, VEGFR-2, VEGFR-3, activating signal transduction to promote angiogenesis, vasculogenesis, and lymphangiogenesis.

**PERICYTE**

Platelet-derived growth factor (PDGF) and its receptor PDGFR-β mediate vessel maturation.

**TUMOR CELL**

Multiple growth factors and receptors activate signal transduction and cell cycle pathways to stimulate tumor cell growth.

**ECDM**

**FLAIR**

**Images courtesy of Massachusetts General Hospital, Department of Radiology**

**Targeting Tumor Angiogenesis**

1. Cancer cells release growth factors that activate endothelial cells during the switch to the angiogenic phenotype. This occurs in response to gene mutations and hypoxia.

2. Growth factors bind to endothelial cell receptors, activating signal transduction pathways and causing cell proliferation.

3. Sprouting vessels secrete matrix metalloproteinases (MMPs) and migrate towards the tumor using specific cell integrins.

4. Glioma cells migrate away from the primary tumor along the outside of host vessels. Promoting tumor cells surround and co-opt host vasculature.

5. Hyperpermeable tumor vessels leak fluid and proteins into surrounding brain tissue, causing vasogenic edema and increased interstitial fluid pressure.

**Targeted Agents**

(Targets shown in diagram above and to the right)

- Bevacizumab (Avastin®)
- Cediranib (AZD2171, Recentin®)
- XL184

**Fluid-attenuated inversion recovery (FLAIR) images**

**Before treatment**

**After treatment**

**Images courtesy of Massachusetts General Hospital, Department of Radiology**

**Vasogenic edema decreases after antiangiogenic therapy**
diagnosed GBM patients received cilengitide (500 mg twice a week) with standard RT plus daily TMZ followed by TMZ (150-200 mg/m²/day x 5 days per month) with cilengitide (500 mg twice a week) for 6 months. This study confirmed that cilengitide could be safely administered with TMZ/RT, and that no unexpected or significant toxicities were attributable to the addition of cilengitide among treated patients. In addition, encouraging evidence of anti-tumor activity was observed, particularly among patients with a methylated methylguanine methyltransferase (MGMT) promoter.

In the second phase 2 study (NABTT 0306), 94 patients with newly diagnosed GBM were randomized to receive standard TMZ/RT plus either cilengitide 500 mg or 2000 mg. The median OS of 18.9 mo. reported for both groups combined compares favorably to the historical benchmark of 14.6 mo. in the phase 3 trial (EORTC-NCIC) of TMZ/RT in newly diagnosed GBM. A randomized phase 3 trial has been initiated to compare standard front-line TMZ/RT against this regimen plus cilengitide in GBM patients with methylated MGMT tumors. In addition, a phase 1/2 study evaluating a dose-dense cilengitide administration schedule is underway among newly diagnosed GBM patients with an unmethylated MGMT promoter.

Another antiangiogenic agent, XL184, an oral TKI of VEGFR-2, hepatocyte growth factor receptor (MET), and c-Kit, was evaluated in a phase 2 study in 42 recurrent GBM patients. Among 26 evaluable patients, 10 (38%) had a best radiologic response of ≥ 50% decrease of enhancing tumor from baseline, including a 100% reduction in one patient. Thirty-five percent had tumor reductions of > 24% to < 49%. VEGF Trap (Aflibercept), a recombinantly-produced fusion protein that captures circulating VEGF and placental growth factor (PIGF), was evaluated in a single-arm phase 2 study in 32 patients with recurrent, TMZ-resistant GBM. Eight of 27 evaluable GBM patients (30%) achieved a PR (50% of patients with relapsed anaplastic glioma were responders). Another agent, CT-322 (Angiocept), a pegylated 94-amino-acid recombinant peptide with high affinity and selectivity for VEGFR-2, is also being evaluated in both recurrent and newly diagnosed GBM patients in ongoing phase 1 and 2 studies.

Most recently, phase 3 trial results for were published for enzastaurin, an inhibitor of both angiogenesis (via inhibition of protein kinase C (PKC)-β, a mediator of VEGFR-2 signaling) and tumor cell proliferation (via targeting of the PI3K/AKT pathway). The open-label trial randomized 266 patients with recurrent GBM to receive either enzastaurin 500 mg daily or lomustine. Despite promising phase 2 response data, enzastaurin performed slightly worse vs. lomustine on all efficacy endpoints in the phase 3 trial: median PFS, 1.5 mo. for enzastaurin vs. 1.6 mo. for lomustine, PFS6, 11.0% vs. 19.0%, and OS, 6.6 mo. vs. 7.1 mo. The disappointing results of this trial, in contrast to other angiogenesis inhibitors, illustrate the complexities of angiogenesis and cell proliferation signaling pathways in GBM, and the inherent challenges in developing effective targeted therapies for this difficult-to-treat tumor.

**Imaging Response to Antiangiogenic Therapy in Glioblastoma**

Among the many challenges confronting clinicians treating GBM patients is how to best measure tumor response to antiangiogenic therapy. Conventional radiographic response using gadolinium MRI is based on a measurement of contrast enhancing tumor (Macdonald criteria), indicating the movement of contrast agent across a disrupted (hyperpermeable) blood brain barrier. Reduction in contrast enhancement observed during anti-VEGF therapy, however, may reflect diminished vessel permeability associated with a normalization of tumor vasculature, rather than a true anti-tumor effect. Indeed, non-enhancing, diffuse, infiltrative disease progression has been documented during anti-VEGF therapy in imaging studies using T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI sequences. In some cases, infiltrative GBM progression has been observed simultaneously with improvement in abnormal gadolinium contrast enhancement (see Figure 2).

To address these concerns, an international GBM working group, Radiological Assessment in Neuro-oncology (RANO), has introduced a new set of imaging guidelines for assessing response and disease progression in the context of antiangiogenic therapy. Among the proposed changes to the Macdonald criteria is to incorporate both enhancing and non-enhancing tumor into the definition of disease progression during antiangiogenic therapy. Clinicians are also awaiting validation of a number of other imaging parameters under investigation as potential biomarkers to predict clinical benefit or recurrence in GBM patients receiving antiangiogenic therapy.

One of these parameters, vascular normalization, has shown some promise as an imaging biomarker in phase 2 studies of cediranib. Using dynamic contrast-enhanced MRI, researchers observed a rapid and dramatic improvement in tumor vessel structure and function—pruning and remodeling of abnormal vessels, decreased vessel diameter and permeability, and thinning of abnormally thick basement membranes—as soon as 1 day after initiation of cediranib therapy. Reduction of vascular permeability, measured by MRI as a decrease in volume transfer constant (Ktrans), correlated with significantly prolonged PFS and OS. Increased cerebral blood volume (CBV) of smaller tumor vessels and a transient increase in plasma collagen IV, which may represent thinning of the abnormally thickened capillary basement membranes, were also predictive of increased OS and PFS, respectively. While hypothesis generating at this stage, the collective parameters of Ktrans, CBV, and plasma collagen IV could comprise a “vascular normalization index” to identify GBM patients most likely to benefit from anti-VEGF therapy early in their treatment course.
Diffusion weighted imaging (DWI) and calculation of apparent diffusion coefficient (ADC), a measurement of cellular water mobility, are also being investigated as potential imaging biomarkers in GBM. High ADC values are thought to correlate with necrotic tumor regions of low cellular density (and high water mobility), whereas low ADC values suggest areas of dense (non-enhancing), higher-grade tumors. Because necrotic tumor is associated with high VEGF expression relative to dense, non-enhancing tumor, baseline ADC could hypothetically predict which patients are more likely to respond to anti-VEGF therapy.

To test this hypothesis, researchers calculated ADC histograms from MRIs of 41 recurrent GBM patients treated with BV and compared them against a control group of 41 patients not treated with BV. BV-treated patients with a low ADCL at baseline had a 2.75-fold reduction in median TTP compared to those with a high baseline ADCL (hazard ratio, 4.1; 95% CI: 1.6, 10.4), while no difference in TTP was seen in control patients with low vs. high ADCL. The median increase in survival was 6.6-fold for BV-treated patients with ADCL ≥ 1201, compared with a 2.4-fold increase for patients with ADCL < 1201, although the difference did not reach statistical significance between the low and high ADCL groups (P=0.33). For BV-treated patients, pretreatment ADC more accurately predicted PFS6 than enhancing tumor volume at first follow-up (73% vs. 58% accuracy, P=0.034). These findings are preliminary and require validation in randomized, prospective trials.

PET imaging using specific tracers that measure tumor metabolism and hypoxia may also offer a more accurate picture of tumor response to antiangiogenic therapy than conventional MRI. This technique was recently evaluated in a pilot study of 21 patients with high-grade gliomas treated with combination BV 10 mg/kg and irinotecan. Tumor metabolism, as measured by PET using fluorothymidine (FLT) uptake, was strongly predictive of OS as early as 1-2 weeks after therapy initiation (P=0.006). MRI radiological response, on the other hand, was only weakly predictive of survival (P=0.06 for both 6-week and best responses). While intriguing, these results also require validation in larger, randomized trials.

Toxicities and Safety Concerns

Antiangiogenic therapies, while generally well tolerated, are associated with a number of adverse effects that necessitate careful clinical monitoring. The most prevalent side effect of anti-VEGF therapy is hypertension, followed by proteinuria, thrombotic events, both arterial and venous, and, less frequently, bowel perforations, delayed wound healing, and congestive heart failure. Despite early concerns about cerebral bleeding with BV therapy, relatively few reports of this complication have surfaced in brain tumor patients receiving VEGF inhibitors, even though CNS hemorrhage can occur spontaneously in GBM patients. In the 167-patient phase 2 study of BV with or without irinotecan, 2 patients (2.4%) who received single-agent BV experienced grade 1 intracranial hemorrhage, and 3 patients (3.8%) who received BV plus irinotecan experienced grades 1, 2, and 4 intracranial hemorrhage, respectively.

Grade ≥ 3 wound healing complications occurred in 2.4% of patients who received BV monotherapy, and in 1.3% of patients who received combination therapy. Two wound dehiscence events were related to craniotomy sites, and 2 patients (2.5%) experienced grade 3 gastrointestinal perforation. Because malignant gliomas secrete large amounts of tissue factor, GBM patients are inherently susceptible to venous thromboembolic events (VTEs), and this susceptibility may be exacerbated during antiangiogenic therapy. In the phase 2 study, the incidence of grade ≥ 3 VTEs was 3.6% for BV and 8.9% for BV plus irinotecan. GBM patients are advised to be physically active, to the extent possible, to lessen the risk for this complication.

Conclusions

Antiangiogenic therapy is dramatically altering the treatment landscape for patients with GBM. The studies described in this review highlight the real progress being made in the application of the antiangiogenic treatment strategy to malignant gliomas. Despite these advances, many significant treatment challenges remain. There is an urgent need to validate predictive biomarkers for accurately stratifying GBM patients and monitoring the efficacy of anti-VEGF treatment. In addition, more work is needed to better understand the biology of both tumor response to antiangiogenic therapy and the mechanisms of tumor escape. Finally, clinicians are encouraged to refer appropriate patients to clinical trials, which provide the best opportunities to take advantage of new treatment approaches that incorporate antiangiogenic therapy.

REFERENCES