

New treatment options in the management of glioblastoma multiforme: a focus on bevacizumab

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Abstract: Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults and carries the poorest prognosis. Despite recent progress in molecular biology, neuro-imaging and neuro-surgical care, the management of patients with GBM continues to harbor significant challenges. Survival after diagnosis is poor even with the most aggressive approach using multimodality therapy. Although the etiology of malignant gliomas is not known, the dependency of tumor growth on angiogenesis has identified this pathway as a promising therapeutic target. Bevacizumab was the first antiangiogenic therapy approved for use in cancer and received accelerated Food and Drug Administration approval for the treatment of recurrent GBM in 2009, the first new drug for this disease in over a decade. This review describes the rationale behind the treatment of GBM with bevacizumab. The pharmacology, efficacy, safety and tolerability of bevacizumab will also be reviewed.

Keywords: glioblastoma multiforme, angiogenesis, bevacizumab

Introduction

Cancers of the brain and nervous system are relatively rare. Glioblastoma multiforme (GBM) continues to be the most common and lethal malignant primary brain tumor in adults.¹ The exact pathogenesis thus far has remained elusive, and most occur in a sporadic fashion.^{2–7} Rarely they occur in the setting of hereditary syndromes.⁸ Despite an aggressive multimodal approach, the median survival time after diagnosis is approximately a year with population-based studies demonstrating even lower median survival rates.^{9,10} Surgery allows histological diagnosis and can provide relief for neurological deficits related to mass effect. Surgery, however, is not curative due to the infiltrative nature of the disease. While only retrospective data are available to evaluate survival benefit, extent of resection correlates with better prognosis.^{11–13} Radiation therapy has been the mainstay treatment for GBM for decades extending median survival to about 9 months versus a median survival of 3 months with no therapy.^{14–18} The role of chemotherapy in gliomas has historically been disappointing, with adjuvant therapy extending longer-term survival in the minority of GBM patients.^{16,17,19} This is in contrast to the more chemosensitive oligodendrogliomas harboring 1p/19q deletions.²⁰ Chemotherapy as standard of care for GBM was only recently established in 2005, when Stupp et al demonstrated that daily temozolomide (TMZ) combined with radiation followed by 6 months of adjuvant monthly cycle TMZ increased median survival by 3 months when compared to radiotherapy alone, and increased 2-year survival from 10% to 26%.²¹ Once disease progression occurs, available salvage chemotherapies are usually unsuccessful, demonstrating a 6-month progression-free survival (PFS-6) of only 15%.²²

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Because of its poor prognosis with current multimodality treatment, a concerted effort is underway to develop new and novel therapeutic strategies that will increase survival and quality of life in patients with GBM. There has been progress in elucidating the molecular changes that underlie the pathogenesis of GBM. GBMs are hypervascular in nature and growth has been shown to be angiogenesis-dependent.^{23,24} Bevacizumab is a humanized monoclonal antibody against vascular endothelial growth factor (VEGF) and has been shown to be an extremely potent inhibitor of angiogenesis. Based on an improved response rate compared to that of historical controls, bevacizumab (Avastin®; Genentech, San Francisco, CA, USA) received accelerated approval by the Food and Drug Administration (FDA) for recurrent GBM in May 2009, thereby becoming the first new drug labeled for gliomas in over a decade. We will review the role of VEGF pathways in glioma angiogenesis and the rationale for bevacizumab in this disease.

Angiogenesis in brain tumors

For tumors to attain a size beyond a few millimeters requires a process known as angiogenesis.^{25,26} Angiogenesis is a physiological process that depends on a well orchestrated balance of angiogenic factors and inhibitors that control the growth of microvessel sprouts via migration and proliferation of endothelial cells.²⁷ When dysregulation of this process occurs, it may provide a suitable milieu for the initiation and maintenance of certain chronic disease states such as psoriasis,²⁸ ocular neovascularization²⁹ and atherosclerosis.³⁰ In addition, pathological angiogenesis has been shown to be a hallmark of certain tumor types such as GBM, colorectal carcinoma, breast and renal cell carcinomas.³¹ Tumor-associated neo-vascularization differs from physiological angiogenesis, characterized by a substantial increase in the proliferation activity of endothelial cells that are structurally “leaky”.³² Tipping the scales in favor of a proangiogenic state requires upregulation of factors such as VEGF-A, platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF2).^{33–35} Additional pathways implicated in tumor angiogenesis are angiopoietin and Notch.³⁶ Enzymes such as metalloproteinase and serine proteinase have been shown to be involved in the induction and suppression of angiogenesis by degrading the extracellular matrix.³⁷ These have all been identified as possible substrates for therapeutic intervention.

Tissue hypoxia resulting from a tumor’s fast exponential growth has been a well-defined trigger of angiogenesis in solid tumors and manifests downstream by a number

of inducible proangiogenic molecular changes. One such example involves hypoxia-inducible factor-1, a transcription factor that regulates the expression of many angiogenesis- and glucose metabolism-related genes and in addition activates the transcription of VEGF in malignant gliomas.^{38,39} VEGF mRNA expression, as well as VEGF receptor expression, are well documented hypoxic induced changes in malignant gliomas.⁴⁰

Various chemokines and mitogens that promote angiogenesis have been shown to be produced by both primary and recurrent gliomas and include basic fibroblast growth factor,⁴¹ interleukin-8 (CXCL8),^{42,43} CXCL12,⁴⁴ and hepatocyte growth factor/scatter factor.⁴³ Neurotrophins and their corresponding receptors, which primarily mediate their effects via the receptor kinases TrkA-C, have been shown to support endothelial cell survival and proliferation as well as neuronal proliferation, differentiation and synaptogenesis.^{45–48} Other mechanisms include endothelial-cell spread and migration in response to certain growth factors mediated by certain integrins,⁴⁹ as well as signaling via stem cell factor and its receptor c-Kit pathway, which is thought to be central in inducing tumor-based angiogenesis.⁵⁰

VEGF-mediated angiogenesis

The first observations of the increased vascular nature of brain tumors were made by Rudolf Virchow during the nineteenth century.²⁶ Later, based upon the concept that tumor angiogenesis was mediated by diffusible factors produced by tumor cells, Folkman proposed that inhibition of angiogenesis would be a reasonable strategy to treat cancer and initiated the isolation of tumor angiogenesis factors.²⁶ In 1983, Senger et al reported the partial purification of vascular permeability factor (VPF), a protein that induced vascular leakage in the skin.⁵¹ In 1989, Ferrara et al isolated VEGF, an endothelial-cell-specific mitogen.⁵² The proteins VEGF and VPF were shown to be one and the same molecule by the work of Connolly et al.⁵³ VEGF and its signaling are important mediators of glioma-induced angiogenesis.

The human VEGF gene has been located to chromosome 6p21.3.^{52,54,55} VEGF has been described as a basic, heparin-binding, homodimeric glycoprotein. There are at least five VEGF glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E) and placental growth-factors PlGF-1 and PlGF-2 that belong within the same family. VEGF-A binding is the dominant mediator of pro-angiogenic signaling in human cancers and VEGF-A has different isoforms that are generated by alternate splicing. The VEGF glycoprotein

acts in a number of ways to mediate the above-described effects. It binds two related receptor tyrosine kinases (RTK), named Flt-1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2)^{56,57} and interacts with a family of co-receptors known as neuropilins. By binding to these receptors, VEGF (and in a similar manner PDGF),⁵⁸ induces homodimerization of two receptor subunits and thereby induces autophosphorylation of the intracellular tyrosine kinase domains.^{56,57,59} This phenomenon then leads to downstream signal transduction. VEGFR-3 has been shown to mediate the mitogenic and survival activity of VEGF via the phosphatidylinositol-3 kinase/phosphatase and tensin homologue/Akt/mammalian target of rapamycin (PtdIns3K/PTEN/Akt/mTOR) pathway,⁶⁰ the PLC γ ⁶¹ and Ras/Raf/mitogen-activated protein kinases MAPK p44/42.^{62–64}

The Notch transmembrane protein and its ligand Jagged/Delta are activated by VEGF signaling and tend to suppress angiogenesis. Blocking the Delta-like ligand 4 has been shown to increase sprouting in a glioma model, paradoxically minimizing tumor growth. Notch signaling seems essential to the negative feedback control of VEGF signaling in brain tumors.^{65–67} In recent years another molecule known as γ -secretase, a presenilin-dependant protease complex also implicated in the pathogenesis of Alzheimer's disease, has been identified as a significant player in the induction and maintenance of tumor-based angiogenesis by cleaving the Notch molecule.^{68,69} VEGFR1 and insulin-like growth factor-1, which are receptors involved in promoting angiogenesis in GBM, have also been shown to be cleaved by γ -secretase.^{70,71}

VEGF has been shown to be a significant regulator of embryonal and physiological and pathological angiogenesis, including that of tumor growth.⁷² In vitro studies have shown that VEGF can promote the growth of vascular endothelial cells derived from both blood vessels and lymphatic vessels,⁷³ act as a survival factor for endothelial cells,^{63,74} induce vasodilatation,⁷⁵ promote inflammation through vascular leakage,⁵¹ induce chemotaxis of endothelial cells,⁷⁶ increase proteolytic enzyme expression and hence promote extracellular matrix degradation,⁷⁶ promote monocyte activation and chemotaxis,⁷⁶ and inhibit the maturation of antigen-presenting dendritic cells.⁷⁷ A number of studies have shown that in addition to endothelial cells VEGF exerts a mitogenic and survival effect on nonendothelial cell types such as nerve cells.⁷⁸

The efficacy of antiangiogenic agents has been demonstrated in preclinical xenograft brain tumor models.^{80,81} Calabrese et al demonstrated that self-renewal capacity of brain tumor cells were maintained by endothelial factors and modulation of this “vascular niche” with antiangiogenic

agents decreased tumor growth.⁷⁹ Because VEGF plays such a significant role in the process of angiogenesis, development of therapeutic interventions targeting VEGF and VEGFR signaling is rational. The use of such agents to treat brain tumors has been increasing and to date there are a number of clinical trials in progress dedicated to this approach, including, the identification of agents that bind specifically to VEGF ligands and those that directly target VEGF receptors.

Bevacizumab

Bevacizumab is a recombinant humanized IgG1 monoclonal antibody (MAb) with an approximate molecular weight of 149 kD. It consists of approximately 93% human and 7% murine sequences.⁸² The antibody itself contains a human IgG1 framework region and the antigen-binding complementarity-determining regions of a murine antibody that binds to VEGF, Mab A.4.6.1. It selectively binds with high affinity ($K_d = 1.1$ nM) and sterically inhibits all biologically active isoforms of human vascular endothelial growth factor to its receptors Flt-1 (VEGFR-1) and KDR (VEGFR-2) on the surface of endothelial cells. By activating these receptors downstream effects include tyrosine phosphorylation and induction of signal transduction pathways involved in mitogenesis and pro-survival activity within vascular endothelial cells.⁸²

Nonhuman safety and toxicology profile

To date there have been no studies assessing carcinogenicity or mutagenicity of bevacizumab. No studies have been conducted to investigate excretion in milk of lactating animals but excretion of IgGs is expected to occur in breast milk. In nonclinical studies using Cynomolgus monkeys it was shown that bevacizumab may impair fertility and that this may be a reversible effect once it has been stopped.⁸³ In addition, when administered at doses of 0.4 to 20 times the weekly human exposure, anatomical pathology revealed several adverse effects on general growth and skeletal development, fertility and wound healing capacity. In rabbits that were treated with bevacizumab there was reduced wound healing capacity.⁸³ Teratogenicity studies performed on pregnant rabbits demonstrated reduced or irregular ossification in the skull, jaw, spine, ribs, tibia and bones of the paws; meningocele; fontanel, rib and hindlimb deformities; corneal opacity; and absent hindlimb phalanges.

Pharmacokinetic profile of bevacizumab

The pharmacokinetic profile of bevacizumab was assessed in humans using an assay that measures total serum bevacizumab concentrations.⁸⁴ Patients who received 1 to 20 mg/kg of

bevacizumab weekly, every 2 weeks, or every 3 weeks, had an estimated half-life of bevacizumab of 20 days, with a range of 11 to 50 days and a predicted time to reach steady state of 100 days. The clearance rate of bevacizumab was influenced by body weight, gender and tumor burden. After correcting for body weight, males had a higher bevacizumab clearance (0.262 L/day vs 0.207 L/day) and a larger Vc (3.25 L vs 2.66 L) than females.⁸⁴

Human safety profile

In the initial clinical studies that led to the FDA approval of bevacizumab in colon, breast, kidney and lung cancers the short-term toxicity of bevacizumab, alone or in combination with chemotherapy, was found to be acceptable.^{85–90} Mild hypertension, manageable by medication, was the most common adverse event in addition to fatigue. A low frequency of more serious adverse events did occur, which included arterial thromboembolic events, congestive heart failure, bone marrow suppression, intracranial hemorrhage, impaired wound healing and gastrointestinal perforations. Toxicities may be potentiated by combining chemotherapeutic agents with bevacizumab; examples of such adverse events include asthenia or fatigue, marrow suppression, neuropathy and liver dysfunction. One relatively uncommon adverse event potentiated by such a regimen is the increased risk of congestive heart failure from 0.5% to 2.2% in patients who have received prior or concomitant anthracyclines. As has been demonstrated in rabbit models bevacizumab impairs wound healing. In a controlled clinical trial in patients with metastatic colorectal carcinoma who underwent surgery, the incidence of wound healing complications, including serious and fatal complications during the course of bevacizumab treatment was 15% and in those who did not receive bevacizumab, was 4%.^{87,88,89} In the clinical trial setting, bevacizumab was administered until at least 28 days after surgery. The appropriate timing of when bevacizumab should be discontinued during the peri-surgical period has not been determined but should take into account the half-life of the drug which is about 21 days with a range of 11 to 50 days. Although there is a potential for immunogenicity no anti-bevacizumab antibodies have been detected thus far.

Bevacizumab and non-CNS solid tumors

Bevacizumab was the first anti-angiogenic inhibitor approved as an anti-tumor therapeutic agent. On February 26, 2004, the FDA approved bevacizumab as first-line treatment for

patients with metastatic colorectal cancer. In a randomized-double, blind-clinical trial of more than 800 patients with metastatic colorectal carcinoma, bevacizumab was compared to the standard chemotherapy of irinotecan, leucovorin (folinic acid) and fluorouracil (IFL). Patients who were given bevacizumab in combination with IFL survived about five months longer and the average time to tumor progression was four months longer than patients receiving IFL alone. The overall response rate to the treatment was 45% compared to 35% for the control arm of the trial.^{88,89} In June 2006, bevacizumab was granted labeling extension for co-administration with 5-fluorouracil-based chemotherapy for the treatment of metastatic colorectal carcinoma based on data from the E3200 trial. This trial was an open-label, randomized, three-arm, active-controlled, multi-center clinical trial in which bevacizumab alone was compared to bevacizumab plus FOLFOX4 (5-fluorouracil, leucovorin, and oxaliplatin) and FOLFOX4 alone. There was a statistically significant improvement in overall survival (OS) in patients receiving bevacizumab plus FOLFOX4 compared to those receiving FOLFOX4 alone.⁸⁷

Bevacizumab received FDA approval in October of 2006 for a labeling extension for patients with unresectable, locally advanced, recurrent or metastatic nonsquamous, nonsmall cell lung carcinoma. This was based on the primary trial E4599, which was a randomized, active controlled, open label, multicenter clinical study evaluating bevacizumab plus carboplatin and paclitaxel versus carboplatin and paclitaxel alone. There was a statistically significant improvement in OS in those receiving bevacizumab with carboplatin and paclitaxel (median OS 12.3 vs 10.3 months; hazard ratio 0.80, $P = 0.013$ stratified log rank test).⁸⁵

An accelerated approval was granted in February 2008 for use in conjunction with paclitaxel in patients with metastatic HER-2 negative breast carcinoma in a single, open-label, randomized, multi-centre study E2100. While response rates were improved there was no improvement in disease-related symptoms nor increased OS.⁹⁰ To date there are no data demonstrating an improvement in disease-related symptoms or increased OS for breast cancer.

Most recently bevacizumab received FDA approval to be used in conjunction with interferon-alfa for patients with metastatic renal cell carcinoma who had undergone nephrectomy. This was largely based on data from the BO17705 trial, a randomized, double-blind, placebo-controlled, multinational clinical trial which demonstrated a median PFS of 10.2 months for the bevacizumab plus interferon arm compared to 5.4 months for the interferon and placebo arm

(hazard ratio [HR], 0.60 (95% confidence interval [CI] 0.49 to 0.72), $P < 0.0001$).⁸⁶ There was no statistically significant advantage in OS.⁸⁶

Bevacizumab and recurrent malignant gliomas

A number of retrospective studies have been published documenting institution experiences with bevacizumab in patients with recurrent malignant glioma (MG) (Table 1). It is difficult to interpret data from these studies due to their retrospective nature. In addition, there often is no distinction between World Health Organization (WHO) grade III and IV tumors. These studies include a variety of combinations of chemotherapeutic agents such as irinotecan, TMZ and carboplatin. Response rates range between 11% to 79%, median progression-free survival (mPFS) from 4.2 to 7.6 months and median overall survival (mOS) from 4.6 to 12.6 months.^{91–96} Ali et al reported a case series

of 13 patients with recurrent heavily pretreated malignant glioma treated with the combination of bevacizumab and irinotecan. Of the thirteen patients nine were started on bevacizumab at a dose of 5 mg/m² every 2 weeks while the rest received a dose of 10 mg/m²; irinotecan was given at a dose of 125 mg/m² every week for 3 weeks. Of the 13 treated patients, 10 (77%) had a radiographic partial response and 3 (23%) had stable disease. The median time to disease progression was 24 weeks while the mOS was 27 weeks.⁹¹ Narayana et al reported on 61 patients with recurrent high-grade gliomas treated with bevacizumab at 10 mg/kg every 2 weeks for 4 doses in an 8-week cycle along with either irinotecan or carboplatin. At a median follow-up of 7.5 months (range 1 to 19 months), 50 (82%) patients relapsed and 42 patients (70%) died of the disease. The mPFS and OS were 5 (95% CI 2.3 to 7.7) and 9 (95% CI 7.6 to 10.4) months, respectively. Radiographic responses were noted in 73.6% of cases.⁹² Norden et al in a retrospective study reviewed 55 consecutive

Table 1 Selected trials of bevacizumab treatment in recurrent malignant glioma

Study	Patients	Regimen	Response rate	mPFS	PFS-6	mOS
Ali et al ⁹¹	13 MG	(9) ^a B 5 mg/m ² q2 wks (4) B 1 mg/m ² + I 125 mg/m ² q3 wks	10 (77%)		mTTP 24 wks	27 wks
Friedman et al ¹⁰¹	167 GBM	(85) B (82) B + I	28.2% 37.8%		42.6% 50.3%	9.2 m 8.7 m
Kang et al ⁹⁵	27 MG	B + I		5.1 m	46%	12.6 m
Kreisl et al ¹⁰³	48 GBM	B 10 mg/kg q2 wks	17 (35%)	16 wks (12–26)	29% (18–48)	31 wks (21–54)
Mohile et al ¹⁰⁵	10 GBM 2 AG	B 10 mg/kg q2 wks + IMRT 6 Gy x5	7 (58%)		76%	
Narayana et al ⁹²	37 GBM 24 AG	B 10 mg/kg q2 wks + I 125 mg/m ² q2 wks or carboplatin AUC6 q4 wks	39/54 (72%)	5 m (2.3–7.7)		9 m (7.6–10.4)
Nghiemphu et al ⁹⁶	44 GBM	B 5 mg/kg q2 wks + various		4.25 m	41%	9.0 m
Norden et al ⁹⁷	33 GBM 22 AG	B 10 mg/kg q2 wks + various	34.1%	23.9 wks (17.7–28.3)	42% GBM 32% AG	35.7 wks (27.7–61.4)
Poulson et al ⁹³	27 GBM 22 AG 3 other	B 10 mg/kg + I q2 wks	30% GBM 15% AG	22 wks	40% GBM 33% AG	28 wks GBM 32 wks AG
Vrendenburgh et al ⁹⁸	35 GBM	(23) B 10 mg/kg + I q2 wks (12) B 15 mg/kg q3 wks + I qwk 4/6 wks	20 (57% (39–74))	24% (18–36)	46% (32–66)	42% (35–60)
Zuniga et al ⁹⁴	37 GBM 14 AG	B 10 mg/kg + I	25/37 (68%) GBM 11/14 (79%) AG	7.6 m GBM 13.4 m AG	63.7% GBM 78.6% AG	11.5 m GBM NA AG

^aNumber of patients.

Abbreviations: AG, anaplastic gliomas; B, bevacizumab 10 mg/kg every 2 weeks; B + I, irinotecan 125–340 mg/m² every 2 weeks; GBM, glioblastoma multiforme; IMRT, intensity modulated radiation therapy; MG, malignant glioma; mTTP, median time to tumor progression; m, months; NA, not available; PFS-6, 6-month progression-free survival; mOS, median overall survival; mPFS, median progression-free survival.

patients with recurrent malignant gliomas who were treated with bevacizumab and chemotherapy (irinotecan, carboplatin, carmustine, temozolomide) to determine efficacy, toxicity, and patterns of recurrence. Only 2.3% of patients had a complete response, 31.8% had a partial response, 29.5% a minimal response, and 29.5% had stable disease. A PFS-6 was 42% for patients with GBM and 32% for patients with anaplastic glioma.⁹⁷ In a retrospective study by Zuniga et al of bevacizumab plus irinotecan in recurrent GBM, 6 (11.8%) of 51 patients discontinued treatment due to a treatment-emergent adverse event, including one with end-stage renal failure and another with gastric perforation.⁹⁴

There are several prospective trials of bevacizumab in recurrent GBM patients. The first study was published by Vredenburt et al.⁹⁸ They reported on a phase II trial of 35 patients divided into two treatment cohorts. The first cohort included 23 patients who received bevacizumab at 10 mg/kg plus irinotecan every 2 weeks. The second cohort included 12 patients who were treated with bevacizumab 15 mg/kg every 21 days and irinotecan on days 1, 8, 22, and 29. The group reported 57% of patients achieving a partial response to therapy and a PFS-6 of 46% (95% CI 32% to 66%). However, 11/35 (31%) patients discontinued therapy due to toxicity and an additional four withdrew due to fatigue. The results were an improvement when compared to historical controls which demonstrate a 6 month PFS of only 9% and response rates of 7% to 9%.^{22,99,100}

Two pivotal trials have documented the bevacizumab monotherapy experience. This included the industry sponsored AVF3708g open-label, multi-center trial¹⁰¹ and a separate independent study, NCI 06-C-0064E, conducted at the National Cancer Institute.¹⁰² The AVF3708g open-label trial included a sample of 167 patients who were randomly assigned to receive bevacizumab alone or in combination with irinotecan 340 mg/m² or 125 mg/m² depending on the use of enzyme-inducing anti-seizure medications. The estimated PFS-6 rates were 42.6% (97.5% CI 29.6% to 55%) for the monotherapy group and 50.3% (97.5% CI 36.8% to 63.9%) for the combined group. While the study was not designed to be comparative, there was no statistically significant difference in survival between the two arms. Objective response rates were 28.2% (97.5% CI 18.5% to 40.3%) for the monotherapy group and 37.8% (97.5% CI 26.5% to 50.8%) for the combined group. The mOS rates were 9.2 months (97.5% CI 8.2 to 10.7 months) for the monotherapy group and 8.2 months (97.5% CI 7.8 to 10.9 months) for the combined group.

In the NCI trial, patients were treated with bevacizumab 10 mg/kg every 2 weeks. Patients who progressed were

offered participation in a companion study where irinotecan 125 to 340 mg/m² was immediately added to biweekly bevacizumab. None of the patients in the NCI trial who were subsequently treated with irinotecan had a response after progression on bevacizumab alone. A response rate of 35% and PFS6 29% (95% CI 18% to 48%) were documented in the first 48 of the 56 patients enrolled into this study. As a result of the data obtained in these trials, bevacizumab received an accelerated FDA approval in May 2009 as monotherapy in patients with GBM who progressed after initial treatment.

The utility of irinotecan in combination with bevacizumab has not been established. This is of little surprise since single agent irinotecan in glioma patients has shown little efficacy in previous studies.^{103,104} To date, no other standard therapy has proven itself to be superior to other treatments when combined with bevacizumab for the treatment of recurrent GBM. As such, various other combinations of bevacizumab have been attempted. Mohile et al reported on a small group of 12 patients who achieved a 58% response rate and a PFS-6 of 76% with fractionated focal radiotherapy on small volume tumors.¹⁰⁵ In an attempt to answer the question of whether the addition of cytotoxic agents may have a synergistic effect, various groups have reviewed their experience with bevacizumab in combination with agents such as TMZ, irinotecan, carboplatin, nitrosureas, etoposide and erlotinib. In a group of 54 patients treated with irinotecan versus 7 patients treated with carboplatin in combination with bevacizumab no significant difference in survival was documented.⁹²

Based on the NCI trial that led to the FDA approval for bevacizumab in recurrent GBM, patients overall tolerated monotherapy well.¹⁰² The most frequently observed severe adverse event possibly or probably related to bevacizumab in 48 treated patients was the occurrence of thromboembolic events which occurred in six patients (12.5%). One patient experienced a stroke and the other three experienced a pulmonary embolus. None of the patients experienced an intracranial hemorrhage. Hypertension was the second most frequent drug-related adverse event that was easily treated with antihypertensive medication. Six patients (12.5%) were removed from the study for drug-associated toxicity that included five thromboembolic events, and one bowel perforation. Grade 1 proteinuria was reported in one patient and Grade 3 hepatic dysfunction was reported in one patient as well. Grade 2 and 3 thrombocytopenia was observed in 1 and 2 patients respectively.

In the AVF3708G trial,¹⁰¹ 98.8 % of patients in the bevacizumab alone arm experienced adverse events with the most common being fatigue (45.2%), headache (36.9%),

hypertension (29.8%), diarrhea (21.4%) and epistaxis (19%). Adverse events took place in all patients in the irinotecan and bevacizumab arm with the most common being fatigue (75.9%), diarrhea (74.7%), nausea (67.1%) and constipation (40.5%). Grade 3 or higher treatment emergent AEs occurred in 46.4% of bevacizumab recipients and 65.8% of bevacizumab plus irinotecan recipients. Selected AEs associated with bevacizumab treatment included arterial thromboembolism (grade ≥ 3 ; bevacizumab, 2.4%; in the irinotecan group, 2.5%), venous thromboembolism (bevacizumab, 3.6%; combined group, 8.9%), and wound-healing complications. Two patients (2.5%) experienced grade 3 gastrointestinal perforation and one patient (1.3%) experienced serious reversible posterior leukoencephalopathy syndrome in the combined group. A grade 1 intracranial hemorrhage was noted in two patients (2.4%) who received only bevacizumab versus 3 patients in the combined group (3.8%) experiencing a grade 1, 2 and 4 respectively. AEs led to bevacizumab discontinuation for four patients (4.8%) in the bevacizumab arm and for 14 patients (17.7%) in the irinotecan group. Two patients in the bevacizumab arm died secondary to neutropenia and pulmonary embolism while one reportedly died of a seizure within the combined group.

Newly diagnosed malignant gliomas

A number of investigators are conducting upfront studies for newly diagnosed GBM since survival benefit data for recurrent disease with the use of bevacizumab have not been established and will not likely be an end-point in future trials for recurrent disease. Nicholas et al reported a study of bevacizumab added to adjuvant TMZ after concurrent chemoradiation.¹⁰⁶ Preliminary results from 42 of 48 enrolled patients demonstrated complete radiographic responses in 5/42 (12%) patients, a partial response in 9 (21%), 13 (31%) were stable and 7 (17%) showed progressive disease.

Lai et al reported a phase II trial of 70 patients treated with focal external beam radiation (60 Gy in 30 fractions), biweekly bevacizumab 10 mg/kg and daily TMZ 75 mg/m². After a two week post-radiation interval, combination therapy with biweekly bevacizumab and monthly TMZ was given. All but 2 patients had total or partial resections. Median follow-up was 17.2 months and 50% of the group had a Karnofsky Performance Scale of 60% to 80%. Median PFS was 13 months (95% CI 11.3 to 15.9 months) and PFS-6 was 89.1% (95% CI 78.6 to 94.7 months) compared to 8.1 months (95% CI 7.0 to 11.7 months) and 64.4% (95% CI 54.5 to 72.7 months) for an internal control group. Median OS was 25 months

(95% CI 16.1 to NA) compared to 21.1 months in the control group. In the treatment group, 6- and 18-month OS was 98.6% (95% CI 90.2 to 99.8 months) and 61.1% (95% CI 45.9 to 73.3 months), respectively, compared to 88.2% (95% CI 80.5 to 93 months) and 60.6% (95% CI 50.8 to 69.1 months) in the control group. Unexpected adverse events included isolated cases of retinal detachment and optic neuropathy.¹⁰⁷ The most common treatment related serious adverse effects included thrombotic complications with 12 (17%) patients being diagnosed with deep vein thrombosis. Hypertension was reported in 8 (11%) patients. Four (6%) patients had wound related infections involving their craniotomy sites. Other adverse events included involvement of the gastrointestinal and renal systems. Neurological events included seizures in 5 (7%) patients, transient ischemic attack/stroke in 3 patients and 1 patient with a traumatic hemorrhage.¹⁰⁷

A similarly designed study of 15 patients was reported by Narayana et al with 1-year PFS and OS being 59.3% and 86.7% respectively.¹⁰⁸ Radiographic responses were noted in 13 of 14 assessable patients (92.8%). Gruber et al utilized a more dose intense schedule of adjuvant TMZ with bevacizumab for newly diagnosed GBM. The PFS-6 survival was 77.5%, the median PFS was 17 months, while the 1- and 2-year OS was 83% and 57%, respectively.¹⁰⁹

Two ongoing randomized phase III trials will investigate the efficacy and safety of adding bevacizumab to standard upfront chemoradiation with temozolomide. A phase III, double-blind, placebo controlled trial (Radiation Therapy Oncology Group 0825) has a target accrual of 720 patients. Patients with newly diagnosed GBM will undergo 3 weeks of standard chemoradiation and in addition will undergo analysis for MGMT promoter methylation and molecular profiling. Patients will then be stratified into two arms for the final 3 weeks of chemoradiation. The first arm will receive concurrent placebo while the second arm will receive biweekly bevacizumab. This will be followed by adjuvant treatment in which the first arm will continue with placebo in addition to TMZ and the second arm will receive TMZ with biweekly bevacizumab.¹¹⁰ The other phase III trial is an industry sponsored randomized, double-blind, placebo-controlled trial with an expected target accrual of over 500 patients. In this trial patients will be randomly assigned to either bevacizumab or the placebo arm, in combination with standard radiation therapy plus temozolomide for 6 weeks. After a 4 week hiatus, patients will continue to receive bevacizumab or placebo, plus adjuvant temozolomide for 6 cycles of maintenance therapy. Bevacizumab or placebo monotherapy will then continue until disease progression.¹¹¹

As yet, the role of bevacizumab in the upfront management of GBM remains to be determined.

Bevacizumab and its clinical benefit

The effects of bevacizumab on vascular permeability are akin to the effects of steroids on cerebral edema.¹¹² We, therefore, have at our disposal another drug other than dexamethasone that can produce improvement of neurological signs and symptoms, which can translate into improved quality of life.¹¹² This was demonstrated in the two trials that led to the FDA approval of bevacizumab in recurrent GBM. In the NCI trial, 50% patients had decreased cerebral edema. Approximately 58% of patients on steroids at the start of treatment were able to achieve an average dose reduction of 59% and 52% had improved neurological symptoms.¹⁰² Findings were similar in the industry sponsored trial which also assessed cognitive function. The majority of patients demonstrated stable performance on a variety of tests at the six week follow-up and 18% to 25% had improved performance.¹¹³ Bevacizumab clearly has a role in the treatment of patients with GBM, independent of survival benefit, for its steroid-sparing effect.

The dramatic effects of bevacizumab on the MRI contrast signal (Figure 1) is at least partially a result of its effect on vascular permeability since reduction in enhancement can be

observed with just one dose of the drug; too short of an interval for true tumorcidal activity. Consequently, MRI contrast signal may not be a reliable proxy marker for extent of tumor as it is for evaluating cytotoxic therapy. For this reason alternative imaging methods are being investigated to assess tumor response such as dynamic susceptibility contrast enhanced MRI and apparent diffusion coefficient measures.¹¹⁴⁻¹¹⁶

Despite the beneficial effect of bevacizumab, concern has arisen that it may actually influence the pattern of disease progression and thereby promote tumor invasion. This was observed in both in vitro and in vivo studies which demonstrated the up-regulation of invasion related genes such as MMP 9 or other pro-angiogenic factors.¹¹⁷ The pattern of relapse, prognosis and outcome of further therapy in patients who failed bevacizumab was recently described in an institutional review. Iwamoto et al reported a series of 37 patients with recurrent GBM who progressed on treatment with bevacizumab. The mOS after progression on bevacizumab was 4.5 months. Seventeen patients (46%) had local recurrence and 6 (16%) had multifocal recurrence. Thirteen patients (35%) had non-enhancing disease progression. The patients with non-enhancing tumor did worse with shorter survival, which was thought to be due to a larger disease burden impacting negatively on performance status. Non-enhancing tumor was also thought to be an independent

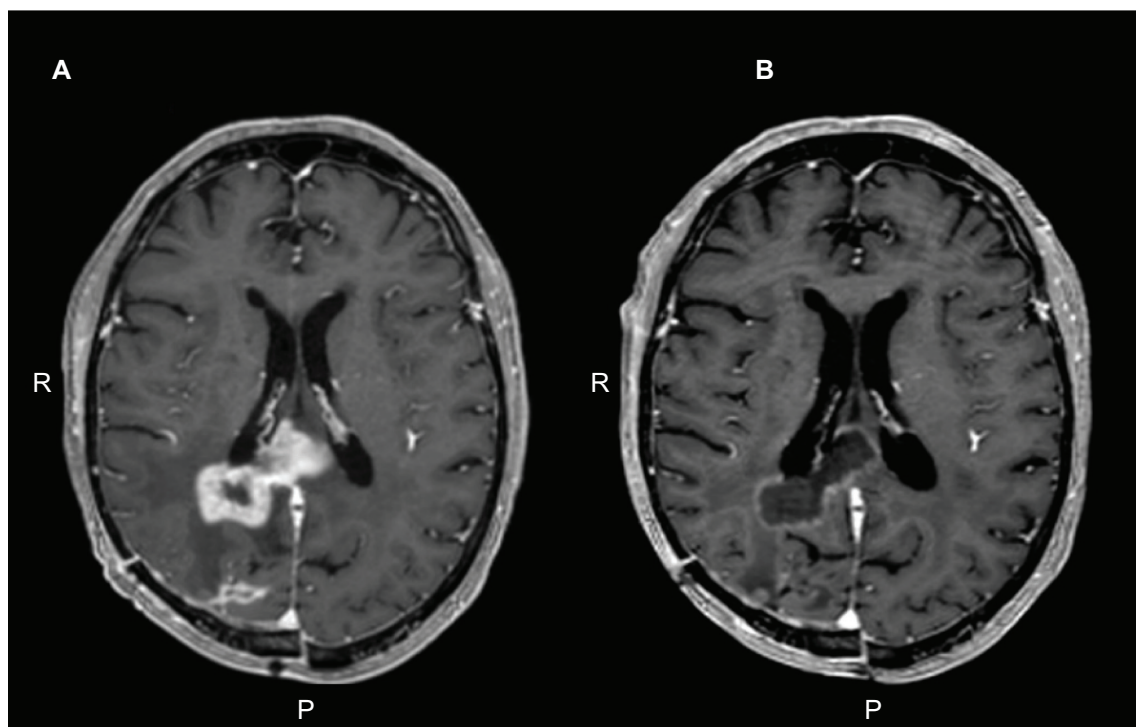


Figure 1. **A)** Axial T1-weighted, post-contrast image of the brain in a patient with glioblastoma multiforme who progressed on temozolomide therapy. **B)** Response after 2 months of bevacizumab monotherapy.

prognostic factor.¹¹⁸ Similar findings were reported by Zuniga et al but others have reported lower rates of infiltrative disease on the order of 20% to 30%.^{92,120} It is difficult to discern whether anti-VEGF therapy actually accelerates or promotes tumor invasion or whether the natural course of the disease is altered so that patients are alive long enough for us to observe this degree of tumor progression, unmasked by the effect bevacizumab has on gadolinium enhancement. It is clear that clinical trials that are using anti-VEGF therapy will need to measure response using methods that incorporate evaluation of non-enhancing disease.

While bevacizumab appears to be an effective agent for recurrent GBM, the majority of patients do not achieve durable disease control and other salvage regimens are required. Often, adding a cytotoxic agent or switching the companion cytotoxic agent is attempted, but efficacy of this practice is unclear. Quant et al described a retrospective review of 54 patients with recurrent MG who progressed on either bevacizumab mono- or combination therapy who were then subsequently treated with an alternate bevacizumab-containing regimen.¹¹⁹ Tumor progression was determined clinically and radiographically. The median prior chemotherapy regimens including the first bevacizumab-containing regimen was 3 (range, 2 to 5). The mPFS on the first bevacizumab-containing regimen was 124 days (95% CI 87 to 154 days); PFS-6 was 33% and the mPFS on the second bevacizumab-containing regimen was 37.5 days (95% CI 34 to 42 days) with a PFS-6 of 2%. In the review by Iwamoto et al 19 of the 37 patients received salvage chemotherapy after failure with bevacizumab. The mOS in those who received salvage treatment was 5.2 months and the PFS-6 was 0%. It is clear that other therapeutic options need to be considered for such patients.¹¹⁸

Conclusion

Tumor angiogenesis has emerged as a valid therapeutic target in clinical oncology and the VEGF system represents a key mediator in this process. While monotherapy with bevacizumab for recurrent GBM has afforded encouraging results, it by no means approaches a cure or durable disease control for the majority of patients. Continued efforts are needed to improve on this early success. Rational combinations of targeted therapy with bevacizumab are appropriately being studied, as well as bevacizumab's role in the upfront treatment of glioma patients. The post-bevacizumab era will prove to be a challenging environment for the neuro-oncology community in evaluating new salvage therapy, but one that for the first time may carry real promise.

Disclosures

The authors disclose no potential conflicts of interest.

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