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REVIEW

A Review of the Treatment Options in Recurrent Glioblastoma: Focus on Bevacizumab

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Abstract: Glioblastoma multiforme (GBM) is the most common primary intrinsic brain tumor in adults. Patients diagnosed with GBM have a median survival of approximately 15 months. Contributing to this poor prognosis is the high recurrence rate of tumors following initial treatment with surgical resection, radiation therapy and chemotherapy. Numerous therapies for recurrent GBM have been proposed, but their safety and efficacy have yet to be demonstrated in definitive clinical trials. Among the more promising treatments, however, is bevacizumab, a humanized mouse monoclonal antibody against vascular endothelial growth factor A (VEGFA) Bevacizumab has antiangiogenic activity and can cause dramatic improvements in tumor size and peritumoral edema as determined by contrast enhanced magnetic resonance imaging. The following is a review of the basic science, translational and clinical studies that have rendered bevacizumab one of the current treatment options for recurrent GBM. Also discussed are the still unanswered questions regarding the use of bevacizumab for this disease.

Keywords: bevacizumab, glioblastoma, therapy, recurrent, malignant glioma

Clinical Medicine Reviews in Oncology 2011:3 79–92

doi: 10.4137/CMRO.S1528

This article is available from http://www.la-press.com.

Introduction

At an incidence of approximately 4 per 100,000 person years, glioblastoma multiforme (GBM) is the most commonly diagnosed adult primary brain malignancy in the United States.[1] Current standard-of-care therapy consists of maximal safe surgical resection followed by radiotherapy with concurrent and adjuvant temozolomide (RT+TMZ), as per the EORTC-NCIC protocol reported in 2005.[2] Relapse, however, remains inevitable and median overall survival in patients under the age of 70 years with good performance status is expected to be less than 15 months.[2]

In contrast to newly diagnosed GBM, there is a lack of consensus about what constitutes the most effective available treatment for recurrent GBM. Options that may be considered include repeat surgical resection, re-irradiation, further cytotoxic chemotherapy, and immunotherapies, usually in the setting of a clinical trial.[3,4] More recently added to this mix are various molecularly targeted agents designed to disrupt key signaling pathways driving glioma cell survival, proliferation, and invasion. Despite highly promising pre-clinical findings, clinical trials of these agents have been generally disappointing as there has been a failure to significantly improve upon the 15% rate of progression-free survival at 6 months (PFS-6) seen historically in ineffective salvage regimens.[5,6]

A possible exception to this is the antiangiogenic agent bevacizumab, which in 2009 gained approval by the United States Food and Drug Administration for use as a single agent in recurrent GBM. Although the accumulated clinical data have been encouraging, much doubt still exists regarding whether this drug brings about robust survival benefits and, if so, in what subgroup of GBM patients and under what conditions. This article aims to review the biological characteristics of bevacizumab, examine the existing clinical evidence for its safety and efficacy, and highlight some of the major challenges investigators face in defining the burgeoning role of bevacizumab in GBM therapy.

Mechanism of Action, Metabolism and Pharmacokinetic Profile

In 1983, Senger et al reported the partial purification of a protein factor from hepatocarcinoma-associated ascites fluid in a guinea pig model.[7] This factor dramatically increased the permeability of vessels lining the peritoneal cavity and was termed vascular permeability factor (VPF). Further characterization of this factor did not occur until 1989, when Ferrara et al reported sequencing the NH2-terminal domain of a protein isolated from bovine pituitary cell-conditioned media, which was found to have potent mitogenic effects on vascular endothelial cells.[8] Subsequent cloning efforts revealed that VPF and this protein are in fact the same molecule, now called vascular endothelial growth factor (VEGF).[9,10]

Five glycoproteins are known to exist within the VEGF family in mammals: VEGFA, VEGFB, VEGFC, VEGFD, and placenta growth factor (PlGF). Of these, VEGFA is the best characterized and has four main isoforms due to alternative splicing.[11] VEGFA-165 is the predominant and physiologically most important isoform, and exerts most of its effects on vascular endothelium through the receptor tyrosine kinase VEGFR-2 (also known as KDR/flk-1).[12] Homo- or heterodimerization of VEGFR-2 after ligand binding leads to autophosphorylation at various tyrosine residues, with Y1175 and Y1214 being the two most important. These phosphorylated residues serve as binding sites for adaptor molecules, which trigger multiple downstream signaling pathways important in regulating cell motility, migration, and proliferation, as well as vascular tone and permeability.[13]

In 1993, a mouse anti-human VEGF monoclonal antibody called A4.6.1 was shown to inhibit the growth of human rhabdomyosarcoma, glioblastoma, and leiomyosarcoma xenografts in nude mice. The antibody had no effect on the growth of the same cell lines in vitro, suggesting that the antitumoral effects of A4.6.1 were due to an inhibition of tumor angiogenesis, rather than a direct cytotoxic effect on tumor cells.[14] Bevacizumab resulted from the successful humanization of A4.6.1 using site-directed mutagenesis of a consensus human IgG1 framework. The humanized antibody showed similar efficacy against VEGF-induced proliferation of endothelial cells compared to A4.6.1 in vitro, and in vivo in nude mice harboring human rhabdomyosarcoma and breast carcinoma xenografts.[15]

Bevacizumab binds to and neutralizes all isoforms of VEGFA, including bioactive proteolytic fragments. Structural studies have demonstrated that the antibody prevents interaction of VEGFA and its

receptors by steric hindrance of key binding residues on VEGFA, rather than by inducing a conformational change in the ligand or by competing for the receptor binding site.[16] Although the VEGFA residues necessary for bevacizumab binding are distinct from those required for high-affinity receptor binding, 9 of the 19 residues involved in the VEGFA-bevacizumab interface are also buried in the interface between VEGFA and its receptor. Moreover, VEGFA binding to bevacizumab appears to resemble a linear peptide sitting within a deep groove, rather than the typical protein antigen-antibody interaction via flat surfaces at the periphery of the complementarity-determining regions of antibodies.[16] These structural attributes provide some insight into why bevacizumab acts as such an effective inhibitor of VEGFA.

In a preclinical study in mice, rats, and cynomolgus monkeys, bevacizumab demonstrated multicompartmental pharmacokinetics similar to other humanized IgG antibodies.[17] As well, the initial volume of distribution after intravenous dosing was consistently smaller than serum volume, suggesting very little distribution outside the intravascular compartment. Clearance occurred in a biphasic manner, with an initial half-life of 1.2 hours in mice, 7 hours in rats, and 11 to 26 hours in monkeys. A terminal half-life of 1 to 2 weeks was observed in all three species. Given that a control non-binding humanized antibody exhibited a similar pharmacokinetic profile, the authors concluded that clearance of bevacizumab likely occurs via a nonspecific antibody clearance mechanism,[17] at least in part mediated by the reticuloendothelial system.[18]

A phase I clinical trial confirmed many of the pharmacokinetic predictions made by allometric scaling of animal data.[19] Maximum serum concentrations achieved were proportional to single intravenous doses infused over 90 minutes. A slight accumulation was observed after serial administrations at days 0, 28, 35, and 42. Clearance kinetics were linear at doses between 0.3 mg/kg and 10 mg/kg, with a half-life of approximately 21 days. As suggested by preclinical studies, the pharmacokinetics of bevacizumab in humans was consistent with limited extravascular distribution, and similar to other humanized monoclonal antibodies with a similar backbone.

Alongside serum levels of bevacizumab, the phase I trial collected data on levels of VEGFA.[19] Interestingly, increased levels of total serum VEGF were found, likely due to an increase in the synthesis of VEGF and/or a reduction in VEGF clearance due to the formation of bevacizumab-VEGF complexes. Serum levels of free VEGF, however, were dramatically reduced after administration of bevacizumab at a dose of 0.3 mg/kg or more, suggesting effective VEGF sequestration.

Preclinical Studies

Preclinical studies on the efficacy of bevacizumab monotherapy focused on nude rodents harboring a wide range of human tumor cell-line derived xenografts.[20] In particular, investigators have directed their efforts on tumors known to overexpress VEGF, which include thyroid, lung, breast, gastrointestinal tract, urinary tract, and female reproductive tract carcinomas.[21] Primary central nervous system tumors known to express high levels of VEGF include glioblastoma, hemangioblastoma, and meningioma.[22]

In 2000, Rubenstein et al reported the first preclinical study of bevacizumab in an animal model of GBM. Bevacizumab was administered intraperitoneally to nude rats harboring xenografts of the human GBM cell line G55 stereotactically implanted in the basal ganglia.[23] Median survival in rats treated on day 1 following tumor implantation was 34.5 days compared to 18.5 days in controls. When treatment was initiated 7 days after tumor implantation, median survival was extended only to 23 days, suggesting greater efficacy at the earlier stages of angiogenesis. Histological analysis revealed reduced vascularity, increased invasive morphology, and a similar size of treated xenografts compared to controls. The authors speculated that induction of tumor hypoxia might promote a more infiltrative phenotype, implying that the use of combination cytotoxic and antiangiogenic therapy might ultimately be required to effect durable responses. Infiltrative cells appeared to be angiogenesis independent, spreading by the co-option of existing host vessels. These features were also reminiscent of cancer stem cells, which may act as a major mediator of bevacizumab resistance.[24]

With this in mind, Mathieu et al recently undertook a study to examine the effects of combining bevacizumab and temozolomide on orthotopic xenografts of human Hs683 and U373 GBM cells in nude mice.[25] Mice receiving combination therapy survived

longer than mice receiving either medication alone. Additive or even synergistic effects achieved using combinations of antiangiogenic and cytotoxic agents may be mediated by several possible mechanisms. Antiangiogenic therapy may normalize the tumor vasculature, improving the delivery of cytotoxic agents.[26] Normalization of the tumor's blood supply may also limit the repopulation of neoplastic cells.[27] Additionally, cytotoxic agents may have antiangiogenic properties that are augmented by VEGF or VEGFR inhibition.[28] Finally, combination therapy may permit distinct cellular populations within a tumor to be targeted, including endothelial cells, cancer stem cells in the perivascular niche, and more differentiated tumor cells.[24,29]

At the same time, concerns have been raised of possible antagonistic interactions between cytotoxic and antiangiogenic agents. Claes et al recently tested vandetanib, a VEGFR2 receptor tyrosine kinase inhibitor, in combination with temozolomide and found indirect evidence, using counts of apoptotic cells within U87 xenografts, of reduced efficacy compared to either vandatenib or temozolomide alone.[30] The authors postulate that normalization of the tumor vasculature as suggested by reductions in the area of enhancement on gadolinium MRI might impede the distribution of temozolomide across the blood-brain barrier. Using microdialysis, Ma et al found a similar effect on intratumoral concentrations of temozolomide by the antiangiogenic agent TNP-470.[31] However, direct evidence in preclinical models of an antagonistic interaction between bevacizumab and cytotoxic chemotherapy has yet to emerge. In the end, whether the delivery of cytoxic agents is impeded or facilitated by antiangiogenic therapy may depend on the balance of proangiogenic and antiangiogenic signals in the tumor microvasculature.[32]

The possibility that anti-VEGF therapy might potentiate the effects of ionizing radiation has also been explored in preclinical models.[33–35] Observing that serum VEGF levels elevate in certain patients with brain tumors after radiation therapy,[36] Gorski et al postulated that endothelial cell killing might be enhanced by anti-VEGF antibodies.[37] They demonstrated a synergistic inhibitory effect on growth of U87 and other xenografts subcutaneously implanted into the hindlimbs of nude mice when VEGF neutralizing antibodies were combined with ionizing radiation. These effects appeared to be mediated mainly by enhanced tumor-associated endothelial cell killing rather than greater cytoxic effects on tumor cells themselves. Similar results under hypoxic conditions were obtained in another study, suggesting that anti-VEGF treatment might compensate for radiation resistance induced by hypoxia.[38]

Clinical Studies in Recurrent GBM

The optimal dose and schedule of bevacizumab administration for the treatment of GBM have yet to be determined. The regimens of 10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks currently being used in many GBM clinical trials were empirically adopted from trials for other cancers. In a phase I/II dose-escalation trial for previously treated metastatic breast cancer, doses of 3, 10 and 20 mg/kg every 2 weeks were administered.[39] The highest response rate was seen at 10 mg/kg, but it was not significantly higher than the other doses. At the higher dose of 20 mg/kg, there was a higher incidence of headache, nausea and vomiting. In a randomized phase II study for metastatic renal carcinoma, doses of 3 and 10 mg/kg every 2 weeks were administered with a significant prolongation of PFS at 10 mg/kg and a trend for improved PFS at 3 mg/kg.[40] For a group of metastatic non-small cell lung cancer patients, 15 mg/kg every 3 weeks was effective at increasing time to progression while 7.5 mg/kg every 3 weeks was not.[41] Lastly, and somewhat conflictingly, a phase II randomized trial for metastatic colorectal cancer found improved time to progression and response rate in an arm receiving 5 mg/kg every 2 weeks but not in the arm receiving 10 mg/kg every 2 weeks.[42] Reasons for inconsistent results might include varying interactions with different combinations of chemotherapy, varying levels of VEGF secreted by different tumor types, and varying levels of VEGF circulating in different patients. Furthermore, the amount of VEGF that must be neutralized to prevent tumor angiogenesis might vary from individual to individual, giving rise to a wider therapeutic window.[43] Overall, it is clear that work still needs to be done to elucidate how to construct efficacious dosing regimens of bevacizumab in a rational manner.

In 2004, Hurwitz et al reported that the addition of bevacizumab to irinotecan, fluorouracil, and leucovorin (IFL) improved median survival in patients with

previously untreated metastatic colorectal cancer from 15.6 months to 20.3 months.[44] As irinotecan (CPT-11) monotherapy had previously shown some modest activity in patients with recurrent GBM,[45,46] there was great interest in likewise using the combination of bevacizumab and irinotecan for recurrent GBM. In 2005, Stark-Vance was the first to report using this approach in a pilot study of 11 patients.[47] Soon thereafter, a small prospective study reported a median time to progression (mPFS) of 2.4 months.[48] Multiple retrospective studies have demonstrated 6-month PFS rates between 17% and 63.7% using this combination approach or bevacizumab monotherapy.[49–56]

Several phase II prospective trials were subsequently mounted. The first, reported by Vredenburgh et al in 2007, examined a total of 35 recurrent GBM patients receiving two different dosing schedules of combination bevacizumab and irinotecan.[57] Bevacizumab was given either at 10 mg/kg every 14 days or 15 mg/kg every 21 days. There were no significant differences in outcome between the two groups, and for the entire cohort PFS-6 and mPFS were 43% and 24 weeks, respectively. This compared favorably with the contemporary benchmark control values of 15% and 9 weeks, respectively.[5] A follow-up phase II trial from the same group, also known as the BRAIN study, was a multicenter, randomized, noncomparative study involving 167 patients assigned to bevacizumab with irinotecan or bevacizumab alone.[58] Patients were given bevacizumab at 10 mg/kg with irinotecan every 2 weeks for cycles lasting 6 weeks in duration. Patients in the bevacizumab arm were given the option of adding irinotecan at disease progression. Patients in both arms were discontinued from treatment at disease progression on combination therapy or after experiencing unmanageable toxicity. Estimated 6-month PFS rates for the bevacizumab-alone and bevacizumab-plus-irinotecan groups were 42.6% and 50.3%, respectively. Median overall survival (mOS) was 9.2 months and 8.7 months, respectively. These results appeared to confirm the findings of Vredenburgh et al suggesting that combination bevacizumab and irinotecan was active against recurrent GBM. Moreover, the favorable objective response rate to bevacizumab alone, and the lack of significantly different outcomes between the study arms, suggested that additional investigation was warranted into using bevacizumab as monotherapy.

Evidence favoring bevacizumab monotherapy was obtained independently by Kreisl et al in a phase II trial conducted on 48 patients at the National Cancer Institute.[59] In this study, patients with documented disease progression after radiation and temozolomide were treated with bevacizumab at a dose of 10 mg/kg every 2 weeks. With further disease progression, irinotecan was added unless it had been used previously without success. Overall, PFS-6 and mPFS were 29% and 16 weeks, respectively, again comparing favorably with historical controls. Overall median survival was 31 weeks and many patients were able to reduce their doses of corticosteroids significantly while on therapy.

On the basis these phase II data, in May 2009 the United States Food and Drug Administration approved the use of bevacizumab as a single agent in patients with recurrent GBM following prior therapy. Since then, additional phase II studies have continued to examine both monotherapy and combination regimens (Table 1). Raizer et al examined the effects of salvage monotherapy with bevacizumab at dose of 15 mg/kg every 3 weeks.[60] For the 50 patients in the trial with histologically confirmed GBM (representing 82% of the study population), PFS-6 at 25% was comparable to the rate of 21% observed in patients receiving salvage therapy with temozolomide,[61] but somewhat lower than the result in the BRAIN study. On the other hand, Kirouz et al achieved results on par with the BRAIN study using the lower bevacizumab dose of 5 mg/kg every 2 weeks with irinotecan in 61 high-grade glioma patients.[62] Gilbert et al recently reported a multicenter study that compared bevacizumab plus irinotecan against bevacizumab plus dose-dense temozolomide.[63] PFS-6 rates in each arm were 39% and 40%, respectively, with similar rates of toxicity for both regimens. Bevacizumab has also been combined with metronomic etoposide,[64] enzastaurin (a PKC-beta inhibitor),[65] cetuximab (a chimeric monoclonal antibody directed against EGFR),[66] and erlotinib[67] in various other recent phase II trials with modest rates of progression-free survival, ranging from 23% to 33% at 6 months. Collectively, these results seem to suggest that the activity of bevacizumab alone may account for most of the improvements compared to historical controls in time to progression. It should be noted however that a well designed randomized clinical trial comparing bevacizumab to placebo control or any other chemotherapy regimen has yet to be reported.

Table 1. Selected clinical trials of bevacizumab for recurrent glioblastoma.

Study	Patients	Regimen	Response rate (%)[a]	mPFS (mo)[b]	PFS-6 (%)	mOS (mo)[b]	OS-6 (%)
Historical (Lamborn)	437	Various	7	1.8	16	6.9	55
Vredenburgh 2007	35	BV+I	57	5.5	46	9.7	77
Friedman 2009	85	BV	28.2	4.2	42.6	9.2	
	82	BV+I	37.8	5.6	50.3	8.7	
Kriesl 2009	48	BV	35	3.7	29	7.1	57
Reardon 2009	27	BV+etoposide	23	4.2	44.4	10.7	
Gutin 2009	20	BV+HFSRT	50	7.3	65	12.5	
Raizer 2010	50	BV	24.5	2.5	25	5.9	54
Sathornsumetee 2010	24	BV+erlotinib	12	4.1	29.2	10.3	
Hasselbalch 2010	43	BV+I+cetuximab	26	3.7	33	6.9	
Moustakas 2010	40	BV+enzastaurin	40	2	23	7.2	60
Gilbert 2010	57	BV+I	28		39		
	60	BV+TMZ	21		40		
Kairouz 2010	61 (HGG)	BV+I	57.5	5.2	40	8.6	67.2

Notes: [a]Response rate is defined as complete response rate plus partial response rate according to the Macdonald criteria; [b]mPFS and mOS results reported in weeks were standardized to months using the following formula: weeks × 84/365.
Abbreviations: mPFS, median progression-free survival; mo, months; PFS-6, progression-free survival rate at 6 months; mOS, median overall survival; OS-6, overall survival rate at 6 months; BV, bevacizumab; I, irinotecan; HFSRT, hypofractionated stereotactic radiotherapy; TMZ, temozolomide; HGG, high-grade glioma.

A therapeutic strategy emerging from preclinical and clinical work in other cancers is the combination of bevacizumab with other molecularly targeted agents. As with other cancers, multiple signaling pathways are co-activated in GBM and the eradication of tumors by inhibition of a single given pathway is unlikely.[68] Possible strategies to overcome this include parallel inhibition of different signaling pathways, as in the combination of bevacizumab and erlotinib, an inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase, which has shown benefits in progression-free survival for non-small cell lung cancer patients;[69] or serial inhibition of different steps in the VEGF pathway, which has been achieved by combining bevacizumab with sorafenib, a tyrosine kinase inhibitor of VEGFR2 and platelet derived growth factor receptor (PDGFR).[70] There are currently separate phase II trials recruiting patients for treatment with bevacizumab in combination with dasatinib (a dual BCR/ABL and Src family tyrosine kinase inhibitor) or temsirolimus (an inhibitor of mTOR). Potentially higher levels of toxicity, however, will present challenges for this approach.[71]

Finally, multimodality strategies are also being actively explored. The combination of bevacizumab and re-irradiation using hypofractionated stereotactic radiotherapy may hold particular promise, with a response rate of 50%, PFS6 of 65% and median overall survival of 12.5 months in one study involving 20 recurrent GBM patients.[72] Other phase II trials are assessing the efficacy of bevacizumab in combination with carmustine wafers.

Simultaneously, efforts are underway to assess the efficacy of bevacizumab in newly diagnosed primary GBM. As these studies are beyond the scope of this review on the treatment of recurrent GBM, they are simply summarized in Table 2.

Salvage Therapy after Bevacizumab

Given the myriad mechanisms of bevacizumab resistance and evasion, it is not surprising that the management of patients who progress on bevacizumab presents a difficult challenge for clinicians. One attempted strategy has been to continue bevacizumab despite progression, while adding a different chemotherapy agent in the hope of achieving a new synergistic effect. Fears that abrupt discontinuation of bevacizumab might produce rebound vasogenic edema have popularized this approach. However, in a retrospective study comprising 54 heavily pretreated high-grade glioma patients who had progressed on a bevacizumab-containing regimen (mostly bevacizumab plus irinotecan), changing to a regimen containing bevacizumab and a different chemotherapy

Table 2. Selected clinical trials of bevacizumab for newly diagnosed glioblastoma.

Study	Patients	Regimen	Response rate (%)[a]	mPFS (mo)[b]	PFS-6 (%)	mOS (mo)[b]	OS-18 (%)
Historical (Stupp)	287	RT+TMZ		6.9	53.9	14.6	39.4
Lai 2010	70	RT+TMZ+BV		13.6	88	19.6	61
Vrendenburgh 2010	125	RT+TMZ+BV+I		14			56
Omuro 2010	25	HFSRT+TMZ+BV	10/13	8.5	87		

Notes: [a]Response rate is defined as complete response rate plus partial response rate according to the Macdonald criteria; [b]mPFS and mOS results reported in weeks were standardized to months using the following formula: weeks × 84/365.
Abbreviations: mPFS, median progression-free survival; mo, months; PFS-6, progression-free survival rate at 6 months; mOS, median overall survival; OS-18, overall survival rate at 18 months; RT, conventional fractionated radiotherapy; TMZ, concurrent and adjuvant temozolomide; BV, concurrent and adjuvant bevacizumab; I, adjuvant irinotecan; HFSRT, hypofractionated stereotactic radiotherapy.

agent (mostly carboplatin) provided little benefit.[55] Median PFS from the beginning of the second bevacizumab-containing regimen was 37.5 days, which was similar to a control group of 11 patients who were salvaged with a non-bevacizumab-containing regimen. The authors concluded that patients who progress on bevacizumab are unlikely to respond to another bevacizumab-containing regimen.

The alternative strategy is to discontinue bevacizumab at progression and commence other experimental salvage therapies. In a review of 37 patients by Iwamoto et al, mOS after progression on bevacizumab was 4.5 months.[73] Nineteen of these patients were tried on a variety of salvage regimens after discontinuation of bevacizumab, the most common of which was a re-challenge with temozolomide. Median OS in this subgroup was 5.2 months, which the authors noted was similar to survival times achieved in recurrent GBM patients in non-bevacizumab protocols. Those who received only supportive care after progression lived a median of 2 months. The authors concluded that good performance patients who have failed bevacizumab treatment are not any more likely to fare poorly on other treatments than bevacizumab-naïve patients, and should be considered appropriate clinical trial candidates.

A third salvage strategy that has been explored is stereotactic re-irradiation, which as discussed previously, has shown some promising efficacy as a treatment for recurrent GBM when given concurrently with bevacizumab. In bevacizumab-naïve patients undergoing salvage treatment, a course of fractionated stereotactic radiotherapy (FSRT) produced a median of 8 months of overall survival and 5 months of progression-free survival in one retrospective study.[74] Similarly, another retrospective study found a median survival was 8.5 months after single-dose stereotactic radiosurgery (SRS) and 7.4 months after FSRT.[75] When applied to patients who had already progressed on bevacizumab, comparable results of 7.2 months OS after FSRT or SRS were obtained in a recent retrospective study on a group of patients with good performance.[76] However, progression-free survival was only 2.6 months. A control group that received a different bevacizumab-containing chemotherapy regimen at progression instead of FRST or SRS achieved a median survival of 3.3 months and a progression-free survival of 1.7 months, which was significantly poorer. These results seem to lend support to the concept that progression on bevacizumab does not necessarily portend a poorer prognosis than progression on other chemotherapy regimens, and that these patients, as long as they have good performance status, should be considered for additional experimental salvage therapy such as re-irradiation.

Safety

Animal studies using 0.4 to 20 times the recommended human dose of bevacizumab predicted adverse effects on: vascularization of the growth plate, resulting in physeal dysplasia;[77] angiogenesis in the female reproductive tract, resulting in reduced ovarian and uterine weights, absence of corpora lutea, and impaired fertility;[78] and the capacity for granulation and re-epithelialization, resulting in reduced tensile strength of healing wounds and delayed wound closure.[79] Additionally, studies on pregnant rabbits given high doses of bevacizumab during the period of organogenesis demonstrated teratogenic effects. These included increased spontaneous abortions,

impaired bone ossification, limb deformities, and fetal cataracts.[79] Human IgG is known to cross the placental barrier and to be excreted in breast milk; thus it has been presumed that fetuses and nursing infants are susceptible to exposure to maternally administered bevacizumab. Bevacizumab is classified category C for pregnancy (uncertain risk) due to the lack of data from human studies but strong evidence of teratogenicity in animals.

Clinically, the use of bevacizumab in adults for GBM in combination with other chemotherapeutic agents appears generally well tolerated. Adverse events may be relatively non-specific or relate directly to the disruption of VEGF function in tissues that require VEGF signaling for normal turnover and repair. In the BRAIN study, the largest prospective randomized study to date employing bevacizumab for GBM, grade 3 or higher adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) occurred at a rate of 46% in patients who received bevacizumab monotherapy.[58] The most common of these were hypertension (8.3% of patients) and convulsions (6.0%). Other grade 3 or higher events relating specifically to the disruption of normal VEGF function included arterial thromboembolism (2.4%), venous thromboembolism (3.6%), and wound-healing complications such as craniotomy dehiscence (2.4%). Two patients in the monotherapy group (2.4%) and 3 in the combination therapy group (3.8%) experienced intracranial hemorrhages. Two patients in the combination therapy group (2.5%) experienced gastrointestinal perforations, a well-known serious adverse event in the colorectal cancer literature. Overall, approximately 11% of patients were discontinued from therapy due to bevacizumab-related toxicity. Three patients died: one patient with a neutropenic infection and one with a pulmonary embolism in the monotherapy group, and one patient with a convulsion in the combination therapy group (Table 3).

In the other two published phase II trials for bevacizumab in recurrent GBM, thromboembolism, hypertension, and bowel perforation were the most common serious adverse events, resulting in comparable rates of therapy discontinuation.[59,60] In addition, Kreisl et al reported 2 patients with grade 3 hypophosphatemia and Raizer et al reported 6 patients with grade 3 fatigue. Neither study reported any adverse event-related deaths (Table 3).

Table 3. Common severe[a] adverse events in patients receiving bevacizumab for recurrent glioblastoma.

Adverse event	Approximate incidence (%)
Fatigue	10
Hypertension	8
Convulsions	6
Venous thromboembolism	4
Intracranial hemorrhage	3
Nephrotic syndrome	3
Gastrointestinal perforation	3
Arterial thromboembolism	2
Wound dehiscence	2

Note: [a]National Cancer Institute common terminology criteria for adverse events grade 3 or higher.

The most common serious adverse effects of bevacizumab generally appear to be manageable. The development of hypertension may be due to the compounding effects of reduced nitric oxide production from blockade of VEGF signaling[80] and a reduction of microvessel density in tissues and organs.[81] In a retrospective study of 154 patients who received bevacizumab in combination with chemotherapy for colon, pancreatic, renal cell, and small cell lung carcinoma, Pande et al found that hypertension of any grade was experienced by 35% of patients.[82] Of these, blood pressure could be controlled within normal range using standard therapy in 85%. Blood pressure monitoring every 2 to 3 weeks (ie, with every dose of bevacizumab according to current regimens) is recommended.[79] Reversible posterior leukoencephalopathy syndrome (RPLS), manifesting with headache, seizures, confusion, lethargy, and cortical blindness, has rarely been reported in association with bevacizumab-related hypertension.[83]

Proteinuria as an adverse effect is thought to be due to inhibition of glomerular endothelial repair, which is mediated by VEGF.[84] Patients with low levels of erythropoietin, which stimulates VEGF release,[85] may be particularly at risk, as well as patients who are being treated with bevacizumab for renal cell carcinoma and those with pre-existing diabetes mellitus.[40] Proteinuria is generally asymptomatic, not more severe than grade 2, and tends to improve after discontinuation of therapy. The incidence of the often

irreversible nephrotic syndrome in patients without other risk factors is probably less than 3%, and 6.5% to 7% in patients with predisposing medical conditions or renal cell carcinoma.[86]

Gastrointestinal perforation or hemorrhage, originally recognized as a serious adverse effect in metastatic colorectal cancer patients,[44] is seen at a fourfold higher incidence (approximately 1% to 2%) in all cancer patients treated with bevacizumab, according to a recent meta-analysis.[87] Most perforations occur within the first 6 months of treatment and may be associated with intra-abdominal inflammatory processes such as gastric ulcer disease, tumor necrosis, diverticulitis, or chemotherapy-induced colitis.[88] Particular caution should be exercised in patients taking non-steroidal anti-inflammatory medications or corticosteroids. Bevacizumab likely causes blood vessel regression leading to weakening of the mucosa and lining of the GI tract, although this remains to be proven in humans.[89] A high level of suspicion for a GI perforation should be maintained when dealing with any patient on bevacizumab complaining of abdominal pain, constipation, and/or vomiting. Because GI perforation is life threatening, emergent surgery should be undertaken as clinically indicated and bevacizumab permanently withheld.[90]

The increased risk of both thromboembolic and hemorrhagic events with the use of bevacizumab presents special challenges to clinicians managing patients with malignant brain tumors. Brain tumor patients have an increased risk of venous thromboembolism (VTE)[91] as well as an increased risk of intracranial hemorrhage, especially in the perioperative period.[92] Antiangiogenic agents are thought to impair the normal renewal of endothelial cells, which exposes subendothelial collagen to the circulation. This might have the dual effect of weakening vessel walls, predisposing to hemorrhage, and triggering platelet activation via the release of tissue factor, predisposing to thrombosis.[93] The expression of high levels of tissue factor by malignant cells is also thought to be one of the main contributors to the prothrombotic state of GBM patients.[94] Currently, there is a lack of evidence supporting the use of anticoagulants such as low-molecular-weight heparin (LMWH) for thromboprophylaxis, although one uncompleted prospective study suggested a trend toward a lower rate of VTE at the cost of a potentially higher rate of major intracranial hemorrhage.[95] In GBM patients with an established diagnosis of VTE, anticoagulation or the placement of an inferior vena cava filter are acceptable treatment options. LMWH may be preferable in this group due to the greater predictability and manageability of the drug's anticoagulant effects compared to warfarin.[96]

Due to fears of an unacceptably high risk of intracranial hemorrhage, GBM patients on anticoagulant therapy for a deep venous thrombosis (DVT) or pulmonary embolism (PE) have generally been excluded from prospective studies on bevacizumab. However, with the recent approval of bevacizumab for patients with progressive GBM and the estimated cumulative annual risk of 30% for VTE in GBM patients,[91] the safety of combining bevacizumab and anticoagulant therapy is likely to be an increasing concern. A recent retrospective study of 282 bevacizumab-treated patients revealed 64 who received concurrent anticoagulation therapy.[97] Of these 64 patients, most of whom were GBM patients treated with enoxaparin for DVT and/or PE, 7 (10.9%) experienced intracranial hemorrhages. This was significantly higher than the rate of hemorrhage in non-anticoagulated patients, which was 0.9%. In another retrospective review of 21 patients who received 5 mg/kg of bevacizumab every 2 weeks while on therapeutic warfarin or enoxaparin, 3 patients experienced intracranial hemorrhages, one of which was symptomatic.[98] Achieving a consensus on this issue will require further investigation, but in the meantime, careful weighing of the relative risks and benefits of bevacizumab therapy in this subset of anticoagulated patients seems advisable.

Clinical Response and Resistance

One of the most immediate benefits of bevacizumab is a reduction in cerebral vasogenic edema in the peritumoral region, which can be seen with as little as a single administration of drug. Consequently, patients are often able to reduce the doses of corticosteroids needed to control edema and manage symptoms. In the BRAIN study, 46.5% of patients in the bevacizumab arm and 67.4% of patients in the bevacizumab plus irinotecan arm experienced a sustained or complete reduction in corticosteroid requirements while on therapy.[99] Other studies have reported that 33% to 72% of patients taking bevacizumab are able

to reduce their dose of steroids significantly.[50,54,57–59] This steroid-sparing effect is thought to account for at least some of the improvements in progression-free survival observed in clinical studies, and is an important benefit independent of the direct effects bevacizumab may have on tumor growth.

The normalization of vascular permeability underlying improvements in cerebral edema correlates with an often rapid and dramatic shrinkage in contrast-enhancing areas of tumor seen on T1-weighted MRI. However, the region of T2 or fluid-attenuated inversion recovery (FLAIR) abnormality, thought to represent the angiogenesis-independent, diffusely infiltrating glioma cell population, might not change concordantly. Norden et al in a retrospective imaging analysis of 55 patients, found that while the area of contrast-enhancement decreased in bevacizumab responders compared to non-responders, the area of FLAIR abnormality progressed in both groups at a similar rate.[54] Other studies examining patterns of progression on bevacizumab identified a higher rate of relapse using

FLAIR abnormality than using contrast enhancement.[56,73] Because CT- and MRI-based methods customarily used to judge response to chemotherapy, such as the Macdonald criteria,[100] utilize only measurements of contrast-enhancing tumor components, clinicians assessing responses to antiangiogenic therapy using these techniques may be misled. There are also other factors unrelated to tumor burden, such as radiologic techniques, postsurgical changes, and seizure activity, which can influence the extent of contrast enhancement.[101,102]

To address limitations of the Macdonald criteria in the age of antiangiogenic therapy, the Response Assessment in Neuro-Oncology (RANO) Working Group recently proposed new response criteria to address the possibility of "pseudoresponse" by incorporating the measurement of non-contrast-enhancing regions of malignant gliomas.[103] Under these criteria, patients on bevacizumab who experience a 25% or more increase in the size of a T2/FLAIR nonenhancing lesion are defined as having progressive disease. Furthermore, patients with increasing T2/FLAIR nonenhancing lesions cannot be classified as having a complete or partial response, or stable disease.

The apparent increased tendency of bevacizumab-treated patients to relapse with diffusely infiltrating, non-contrast-enhancing disease has raised important questions about the effect of antiangiogenic therapies on glioma cell biology. Reported increases in progression-free survival without accompanying increases in overall survival time suggest that tumor growth is slowed transiently, during which time cancer cell adaptation occurs, and then ultimately evasion. Paez-Ribes et al recently conducted a series of experiments in mice in which orthotopically implanted GBM xenografts treated with a VEGFR kinase inhibitor were compared against xenografts derived from VEGF knocked-out GBM cells.[104] Mice implanted with VEGF knocked-out GBMs experienced significantly prolonged survival whereas the inhibitor-treated mice benefited marginally. In both cases, but particularly in VEGF knocked-out GBMs, increased perivascular tumor cell invasion was noted in association with larger areas of hypoxia. These results echo the previously discussed findings of Rubenstein et al using bevacizumab itself,[23] and hint that anything less than a complete absence of VEGF signaling within a tumor is insufficient to counterbalance unwanted adaptive changes to a more invasive phenotype, and is ultimately insufficient to produce an overall survival benefit.

Patients on bevacizumab therapy may also relapse with contrast-enhancing disease or may not respond at all. To account for these distinct patterns of progression, Bergers et al has described the existence of at least four mechanisms of evasive resistance to antiangiogenic therapy, based on available experimental evidence.[105] First, tumors may begin to express alternate pro-angiogenic factors, including fibroblast growth factor 1 and 2, ephrin A1 and A2, and angiopoietin 1, which allow reestablishment of the tumor vasculature. Second, tumor hypoxia may trigger the recruitment of pro-angiogenic bone marrow-derived cells that can differentiate into various elements of the tumor vasculature such as endothelium and pericytes, or can release angiogenesis-promoting cytokines, growth factors, and proteases.

Third, pericytes may serve to protect a subset of tumor vessels through juxtacrine VEGF signaling that is concealed from anti-VEGF therapy. Fourth, VEGF signaling may be a suppressor of a pro-invasive,

angiogenesis-independent phenotype in some tumor cells, which becomes activated with anti-VEGF therapy. Any of these mechanisms may actively pre-exist in some tumors, providing a possible explanation for the subset of patients who do not show even a transitory response to anti-VEGF therapy.

Practical Concerns

Glioblastoma patients embarking on antiangiogenic therapy with bevacizumab should be aware of the logistical implications of intravenous drug administration every 2 weeks, possibly for the remainder of their lives. This may have a significant negative impact on quality of life for some patients. Furthermore, at an estimated cost of $9,000 USD per month for the drug alone, the answers to the questions of which patients are likely to benefit most from treatment, and when during the course of disease is treatment best initiated, will undoubtedly have major economic repercussions for patients and their health insurance providers. For example, the use of concurrent and adjuvant bevacizumab up to the completion of 6 cycles of adjuvant temozolomide in the EORTC-NCIC protocol for newly diagnosed GBM patients mandates at least 15 doses.

Predictive markers, both imaging-based and tissue-based, may help improve the way patients are selected for antiangiogenic therapy and address mounting concerns of cost-effectiveness. Recent work has demonstrated that hypointensity on pre-treatment susceptibility-weighted MRI within the region of contrast-enhancement, a sign of damaged vasculature, may be predictive of PFS and OS.[106] Other work in diagnostic imaging has suggested that parameters on both perfusion- and diffusion-weighted MRI may also have utility in predicting responses to antiangiogenic therapy.[107,108] Immunohistochemical studies on surgical specimens have identified the overexpression of VEGF to be associated with a higher likelihood of radiographic response, and high carbonic anhydrase 9 (CA9) expression to be associated with poorer survival.[109] However, a more recent study prospectively examining a large panel of angiogenic and hypoxic markers, including VEGF, CA9, GLUT-1, and HIF1-alpha, failed to confirm that any of these were predictive of radiographic response or survival.[110] Clearly, there continues to be a great need for reliable methods of identifying the subgroup of GBM patients that is mostly likely to respond to antiangiogenic therapy.

Other practical concerns have been highlighted by the use of bevacizumab with other medications and interventions commonly used in GBM patients. Given its clearance from the body through the reticuloendothelial system,[18] particular attention has been paid to whether interactions might exist with liver enzyme-inducing antiepileptic drugs. In a phase II safety and efficacy study, no differences in response rate, PFS, or overall survival were seen when combination bevacizumab and irinotecan was given to patients on enzyme-inducing antiepileptic drugs versus those on non-enzyme-inducing antiepileptic drugs.[111] Thus, it is thought that dose adjustments for patients on these medications are generally unnecessary. Neurosurgeons must also consider the timing of bevacizumab administration in relation to surgical interventions patients might need, such as repeat tumor resection or the insertion of ventriculoperitoneal shunts or Ommaya reservoirs. Due to interference with mechanisms of wound healing, it is recommended to discontinue bevacizumab at least 28 days prior to elective surgery, and to wait at least 28 days or until surgical wounds are fully healed before starting bevacizumab.[79]

Finally, patients considering bevacizumab therapy should be counseled frankly about the current lack of data supporting an overall survival benefit. Although increases in progression-free survival usually equate with improvements in quality of life, these may come at the risk of more aggressive and infiltrative disease at relapse. Further salvage options in such situations are at present extremely limited.

Conclusion

From the sheer number of clinical studies on the topic published in the last 3 to 4 years, it is evident that bevacizumab has generated considerable excitement in the neuro-oncology community. Its rational basis in glioma biology, relatively low level of toxicity, and promising improvements in progression-free survival in early-phase clinical studies all seem to justify the optimism that a new weapon in the difficult war against glioblastoma might be at hand. At the same time, this optimism should be tempered by the sobering fact that less than half of patients with

recurrent GBM are expected to experience a complete or partial response on bevacizumab. The great majority of those who do respond will relapse in less than 6 months, and what distinguishes those who respond from those who do not is unknown. Future work must elucidate the mechanisms of bevacizumab resistance in order to develop strategies against the infiltrative, angiogenesis-independent component of gliomas that unfailingly make them incurable. Work on radiographic and molecular biomarkers will also help improve the selection of patients for bevacizumab and other targeted agents. Approaches combining cytotoxic therapies with targeted agents tailored to the molecular characteristics of individual patients' tumors will likely offer the greatest chance of achieving meaningful gains in survival in GBM patients in the foreseeable future.

Acknowledgement

This work was supported by the Intramural Research Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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