Antiangiogenic Strategies for Treatment of Malignant Gliomas

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Summary: Numerous antiangiogenic agents with diverse mechanisms of action are currently under investigation for the treatment of patients with glioblastoma (GBM), a diagnosis that continues to carry a poor prognosis despite maximal conventional therapy. Early clinical trials suggest that antiangiogenic drugs, which target the blood vessels of these highly angiogenic tumors, may have clinical benefit in GBM patients. Antiangiogenic agents have potent antiedema and steroid-sparing effects in patients, and emerging data suggest that these drugs may modestly improve progression-free survival. Although these early results are encouraging, several issues arise regarding the use and efficacy of these agents. Interpretation of the radiographic changes that occur after treatment with antiangiogenic agents presents a major challenge. Still lacking are reliable radiographic and biologic markers that can predict which patients will benefit from treatment and that accurately indicate response and progression during therapy. In addition, most patients treated with antiangiogenic drugs eventually progress, and the mechanisms by which tumors escape from therapy are only beginning to be understood. Larger prospective trials that incorporate correlative biomarker studies will be required to address these challenges. Here, we summarize the clinical experience with antiangiogenic therapy in patients with malignant gliomas (MG), review the major issues concerning the use and development of these agents, and discuss strategies that may build upon the initial gains observed with antiangiogenic agents. **Key Words:** Malignant glioma, glioblastoma, angiogenesis, vascular endothelial growth factor, edema, biomarker.

INTRODUCTION

With current standard therapy, the prognosis for patients with newly diagnosed malignant glioma (MG)—that is, WHO grade III gliomas (anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic oligoastrocytoma) and WHO grade IV glioma or glioblastoma (GBM)—remains unsatisfactory.[1] The standard treatment for GBM (which is the most common and aggressive malignant primary brain tumor in adults) consists of maximal safe surgical resection, radiation, and temozolomide chemotherapy and results in a median overall survival (OS) of less than 15 months.[2] For patients with MG who progress through standard therapy, there is no established treatment, and these patients typically survive less than 6 months.[3] Furthermore, patients with MG experience neurological deficits and considerable morbidity as a result of vasogenic edema induced by tumors. Corticosteroids, the most commonly used agents to treat edema in brain tumor patients, are associated with considerable adverse effects.[4,5] In recent years, however, therapeutic strategies directed at the blood vessels that supply tumors have demonstrated promise in improving the outcomes of patients with MG.

The discovery that tumor progression depends on angiogenesis (i.e., on the growth of new blood vessels from pre-existing vessels) led to the development of antiangiogenic therapy for cancer.[6–10] This treatment paradigm has been proven beneficial in phase III clinical trials of several advanced solid tumors, and three antiangiogenic drugs are now approved by the U.S. Food and Drug Administration (FDA) for use in cancer patients. Bevacizumab (Avastin; Genentech, South San Francisco, CA), a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), is approved for first-line therapy in combination with conventional chemotherapy in patients with advanced colorectal, non-small cell lung, and breast cancer.[11–13] Sorafenib (Nexavar, Bayer, Leverkusen, Germany) and sunitinib (Sutent;

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Pfizer, New York, NY) are two tyrosine kinase inhibitors (TKI) targeting the VEGF receptor (VEGFR) that are approved for use as single agents in cancer therapy. Sorafenib is approved for use in patients with advanced renal cell and hepatocellular carcinoma[14,15]; sunitinib is approved for treatment of advanced renal cell carcinoma, as well as for progressive gastrointestinal (GI) stromal tumors.[16,17]

Growth of MG is also dependent on angiogenesis, and VEGF appears to play a primary role in the development and function of new tumor vessels.[18–20] A large body of preclinical evidence suggests that antiangiogenic therapy may be effective in treating MG,[21,22] and preliminary clinical data suggest that antiangiogenic agents have a beneficial effect in patients with MG.[23,24] Recently, the FDA Oncologic Drugs Advisory Committee voted unanimously that the response seen with bevacizumab in a recent phase II trial of patients with recurrent GBM[25] is of sufficient magnitude to be reasonably likely to predict clinical benefit. As a result, the FDA granted accelerated approval for bevacizumab as a single agent for recurrent GBM patients. FDA approval of bevacizumab will naturally have major implications regarding the management of patients with recurrent GBM. Nonetheless, several issues regarding the clinical use and efficacy of angiogenesis inhibitors in MG are emerging, such as the lack of valid biomarkers of response or progression and the emergence of resistance.[26–28] In this review, we focus on the current progress of clinical trials of antiangiogenic therapy in MG (FIG. 1) and on emerging issues in the clinical development of such therapy.

VEGF PATHWAY INHIBITORS

Because of its prominent role in MG tumor angiogenesis, the VEGF pathway was rapidly identified as an attractive therapeutic target.[18–22] Thus, agents that target the VEGF pathway have become the most clinically advanced antiangiogenic drugs. Several VEGF-targeting approaches are under clinical investigation in MG, including strategies that target VEGF and VEGF receptors (Table 1).

VEGF inhibitors

Bevacizumab and the soluble decoy VEGF receptor aflibercept (VEGF-Trap; Regeneron, Tarrytown, NY) are VEGF-sequestering molecules characterized by long half-lives and high specificity. Several reports published over the past few years have suggested that bevacizumab may be beneficial for recurrent MG patients.

In the first reported prospective study of an anti-VEGF therapy in MG patients, high radiographic response (complete or partial response as defined by standard Macdonald criteria)[29,30] and 6-month progression-free survival (PFS6) proportions were observed with the combination of bevacizumab and irinotecan.[31,32] In this phase II clinical trial, 68 patients (33 recurrent anaplastic glioma and 35 recurrent GBM) were treated with bevacizumab and irinotecan in two cohorts. The radiographic response proportions of 57% for recurrent GBM and 61% for anaplastic glioma patients observed[33] compared favorably with response proportions seen with temozolomide at first recurrence (5% for recurrent GBM[34] and 35% for recurrent anaplastic glioma).[35] Responses were also associated with neurological improvement and reduction or discontinuation of corticosteroid requirements, indicating that a clinical benefit was derived from treatment. Furthermore, durability of the responses was suggested by an increase in the PFS6 proportions (43% for recurrent GBM patients and 59% for AG patients)[33] over historical benchmarks (15% and 31%, respectively).[3] Although treatment was generally well tolerated, toxicity was observed; 11 of the 35 recurrent GBM patients (31%) discontinued therapy due to treatment-related events. There were thromboembolic complications in 8 of the 68 patients overall (12%), including one arterial stroke, and 2 patients (3%) had CNS hemorrhages.[33] Other toxicities included proteinuria, fatigue, and GI toxicity. Of note, radiographic response and PFS6 proportions were similar in the two treatment cohorts; however, the cohort with increased irinotecan dosing was associated with greater GI toxicity.

Another prospective study of 21 recurrent MG patients, of whom 17 had GBM, reported a comparably high MRI response proportion of 36% with the combination of bevacizumab and irinotecan.[36] Additionally, several retrospective studies have reported similar findings with bevacizumab in recurrent MG patients. Radiographic response proportions between 35% and 50% have been observed with the combination of bevacizumab and conventional chemotherapy in several case series, with responses often occurring rapidly after treatment initiation.[37–40] These studies also reported delayed tumor progression, suggesting that clinical benefits were derived. One study reported that six-month progression-free survival was 42% for recurrent GBM patients, and an apparent antiedema effect of bevacizumab was evident, in that 33% of patients reduced their corticosteroid requirements.[39]

Treatment was generally well tolerated in these retrospective series, although thromboembolic and hemorrhagic complications were reported. Thromboembolic events occurred in 5 of 44 patients (11%)[39] and in 7 of 77 patients (9%)[40] in the two larger studies. Notable adverse events included six pulmonary embolisms, one superior mesenteric vein thrombosis, and one myocardial infarc-

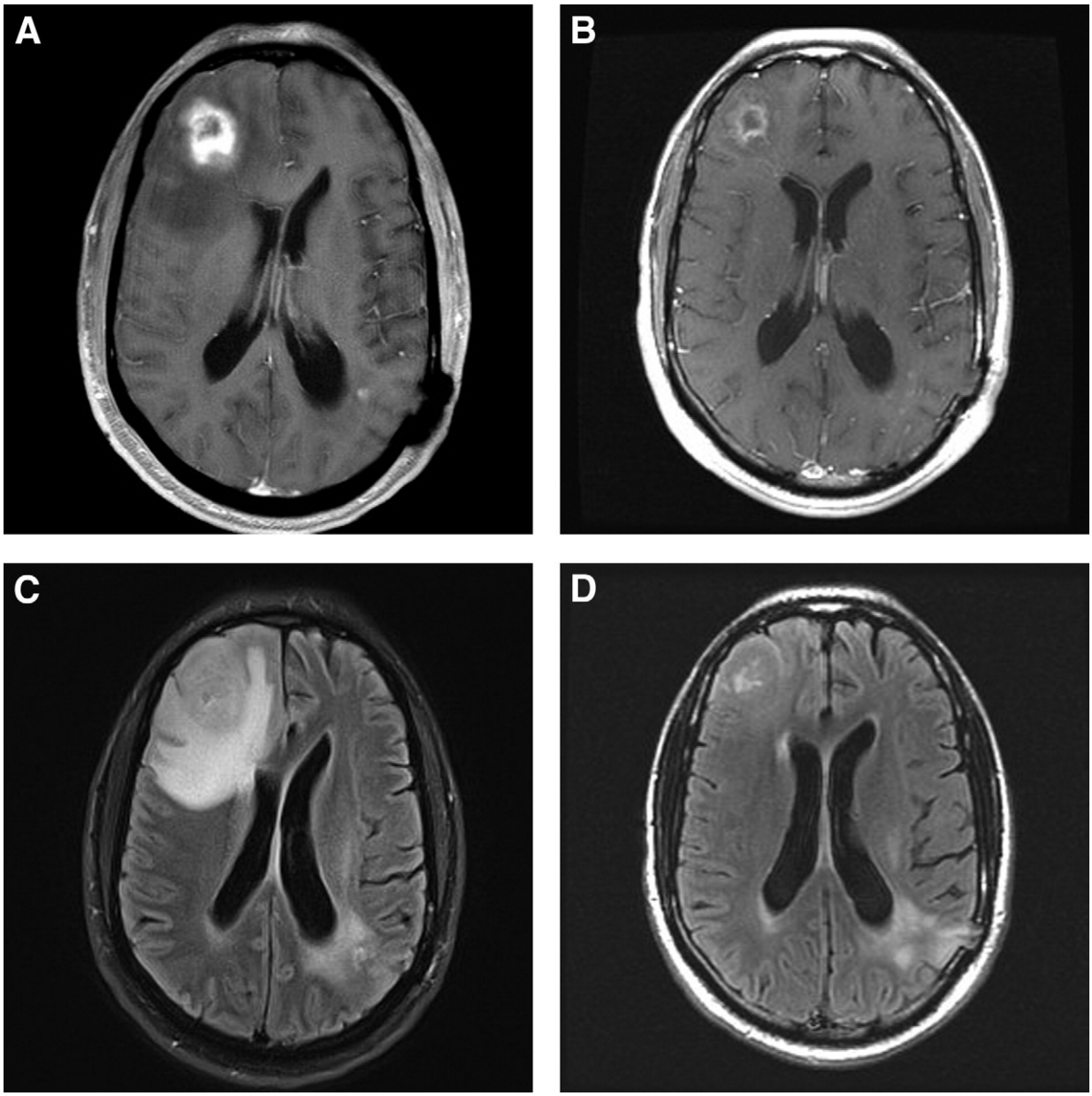

FIG 1. Axial T_1-contrast enhanced MRI of a 40-year-old man with a multifocal recurrent glioblastoma (A) before and (B) 8 weeks after treatment with XL-184 (VEGFR and Met inhibitor) showing a significant decrease in enhancement. In the same patient, axial FLAIR MRI (C) before and (D) after treatment shows the reduction in edema that correlated with symptomatic improvement.

tion (MI). Intratumoral hemorrhages were observed in 5 of 77 patients (6.5%) in one series[40], and two asymptomatic intracranial hemorrhages were noted in a series of 44 patients.[39] Other adverse events of note included two GI perforations[37,39] and one case of reversible posterior leukoencephalopathy syndrome.[40]

All of these studies were of bevacizumab in combination with cytotoxic chemotherapy. Bevacizumab was most often combined with irinotecan in these studies, and irinotecan is known to have minimal efficacy in recurrent MG patients.[41] How much chemotherapy contributed to the effect of bevacizumab and whether bevacizumab possessed single-agent activity in recurrent MG patients remained unanswered. To address these questions, a phase II trial of 167 recurrent GBM randomized the patients to receive either bevacizumab alone (10 mg/kg every 2 weeks) or in combination with irinotecan.[25] The radiographic response and PFS6 proportions of 37.8% and 50.3%, respectively, observed with combination therapy were similar to the proportions seen with bevacizumab alone (28.2% and 42.6%, respectively).[25] In addition, the median OS for the combination therapy (8.7 months) was similar to that observed with bevacizumab alone (9.2 months). In this study, most patients were able

Table 1. *Selected Antiangiogenic Agents and Their Potential Targets Currently in Clinical Trials for Adult Malignant Glioma*

Agents	Targets	Mechanism
VEGF inhibitors		
Aflibercept	VEGF-A,B, PlGF	Decoy receptor
Bevacizumab	VEGF-A	Monoclonal antibody
VEGF receptor inhibitors		
Cediranib	VEGFR1–3, PDGFRβ, c-Kit	Tyrosine kinase inhibitor
CT-322	VEGFR1–3	Fibronectin (adnectin)-based inhibitor
Pazopanib (GW786034)	VEGFR1–3, PDGFRβ, c-Kit	Tyrosine kinase inhibitor
Sorafenib	VEGFR2,3, BRAF, PDGFRβ, c-Kit, Ras, p38α	Tyrosine kinase inhibitor
Sunitinib	VEGFR2, PDGFRβ, Flt3, c-Kit	Tyrosine kinase inhibitor
Vandetanib (ZD6474*)	VEGFR2, EGFR, RET	Tyrosine kinase inhibitor
Vatalanib (PTK787)	VEGFR1–3, PDGFRβ, c-Kit	Tyrosine kinase inhibitor
XL-184	VEGFR2, Met, RET, c-Kit, Flt3, Tie-2	Tyrosine kinase inhibitor
Non-VEGF pathway inhibitors		
ABT-510	CD36 receptor	Thrombospondin-1 mimetic peptide
AMG 102	HGF/SF	Monoclonal antibody
Brivanib	FGFR, VEGFR2	Tyrosine kinase inhibitor
Lenalidomide	FGF pathway	Immunomodulatory and anti-inflammatory
Dasatinib	PDGFRβ, Src, BCR-ABL, c-Kit, EphA2	Tyrosine kinase inhibitor
Imatinib	PDGFRβ, BCR-ABL, c-Kit	Tyrosine kinase inhibitor
Tandutinib (MLN518)	PDGFRβ, Flt3, c-Kit	Tyrosine kinase inhibitor
Celecoxib	COX-2	Suppress VEGF and FGF
2-methoxyestradiol (2ME2†)	HIF-1α	Disrupt tumor microtubules and suppress HIF-1a
Metronomic temozolomide	Endothelial cells, EPCs	Induce apoptosis, inhibit recruitment of EPCs
Endothelial cell migration inhibitors		
ATN-161	Integrin $\alpha5\beta1$	Fibronectin-derived PHSRN peptide
Cilengitide	Integrins $\alpha v\beta3$, $\alpha v\beta5$	RGD synthetic peptide

BCR-ABL = a gene fusion protein product associated with chronic myeloid leukemia; COX = cyclooxygenase-2; EGFR = epithelial growth factor receptor; EPC = endothelial progenitor cell; FGF = fibroblast growth factor; FGFR = fibroblast growth factor receptor; Flt3 = fms-related tyrosine kinase 3; HGF/SF = hepatocyte growth factor/scatter factor; HIF-1α = hypoxia inducible factor 1α; PDGFR = platelet-derived growth factor; PlGF = placental growth factor; RA = retinoic acid; RT = radiotherapy; TMZ = temozolomide; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor.
*Trade name Zactima (Astra Zeneca). †Trade name Panzem (EntreMed).

to reduce their corticosteroid dose by at least 50%, and treatment was well tolerated; intracranial hemorrhages were noted in 3 of the 167 patients.

Another recently reported phase II study evaluated the benefit of bevacizumab monotherapy in recurrent GBM patients.[42] In 48 heavily pretreated patients, the authors observed a 35% radiographic response proportion and a PFS6 proportion of 29%, values similar to those observed in the bevacizumab monotherapy arm of the randomized phase II trial described above. Furthermore, when irinotecan was added to bevacizumab at progression in 19 patients, there were no objective radiographic responses. Eighteen of these patients (95%) experienced disease progression by the second cycle, and the median progression-free survival (PFS) was 30 days. These two studies suggest that irinotecan adds little, if any, benefit to bevacizumab in recurrent MG patients.

Taken together, the studies of bevacizumab in recurrent MG patients indicate that bevacizumab may have clinical efficacy in this population, with a moderate but acceptable toxicity profile. The clinical utility of bevacizumab has mainly manifested as an antiedema effect and an increased PFS; however, an overall survival benefit in GBM patients has not been definitively demonstrated. Although it has been shown that PFS6 is strongly associated with OS at 12 months in recurrent GBM trials,[43] recent survival data of antiangiogenic agents in recurrent MG patients are conflicting. A median OS of 9 months was observed in a recent prospective study of 61 recurrent MG patients treated with bevacizumab and chemotherapy, which the authors contended was higher than comparison historical rates of 3 to 6 months.[44] However, both recurrent anaplastic glioma and GBM patients were combined in their survival analysis. In a recent retrospective analysis of recurrent GBM patients, the median OS of patients treated with antiangiogenic agents (39 weeks) was nearly identical to the median OS rate of patients treated with cytotoxic chemotherapies (37 weeks).[45] Thus, it is unclear whether angiogenesis inhibitors confer a survival advantage in MG patients, and prospective,

controlled trials are needed to address this question. Because clinical data currently indicate that any potential survival benefits with antiangiogenic agents will likely be on the order of months, many investigators are combining bevacizumab with various chemotherapy and molecularly targeted agents in recurrent MG patients to try to gain efficacy (Table 2).

Aflibercept is a soluble VEGF decoy receptor that consists of a VEGF receptor fused to an immunoglobulin constant region. It has a VEGF binding affinity several hundred times greater than bevacizumab and the capacity to also bind the VEGF family members VEGF-B and placental growth factor (PlGF).[46] An ongoing phase II clinical trial of 48 recurrent MG patients treated with aflibercept monotherapy reported response proportions of 50% for anaplastic glioma and 30% for GBM patients,[47] values similar to the response proportions observed in bevacizumab trials.[47] There was moderate toxicity, however, and treatment was discontinued in 12 patients (25%), on average less than 2 months into therapy.

VEGF receptor inhibitors

Currently many VEGFR-targeted TKIs are in clinical trials for MG (Table 1). These small molecule inhibitors were initially developed as specific inhibitors of the VEGFR tyrosine kinase domain; most, however, have the capacity to inhibit many other tyrosine kinases such as the platelet-derived growth factor receptor (PDGFR), Raf, and c-Kit.[48] Although this lack of specificity may result in more off-target effects, the potential for simultaneous inhibition of several proangiogenic or pathogenic signaling pathways in MG may be a favorable feature of these agents.

Cediranib (AZD2171, Recentin; AstraZeneca, London, England) is a potent pan-VEGFR TKI with modest activity against PDGFR and c-Kit. In a recent phase II trial of cediranib monotherapy in recurrent GBM patients, radiographic responses were observed in 9 of 16 patients (56%), and 26% of patients were alive and progression-free at 6 months.[49,50] There was also a modest improvement in median OS in cediranib-treated patients relative to a historical database (211 days *vs* 175 days). Furthermore, an antiedema effect was detected with cediranib monotherapy. Using advanced MRI techniques, the authors were able to quantify the reduction in vasogenic edema resulting from cediranib therapy, and corticosteroid requirements were discontinued or decreased in 15 of 16 patients. Toxicity was moderate. Although only 2 of 31 patients were removed from the study because of toxicity, and no treatment-related deaths or intracranial hemorrhages occurred, a high frequency of hypertension was observed, and most patients required the addition of at least one antihypertensive drug. Fatigue and diarrhea were also frequent toxicities. As a result of this study, a phase II study of cediranib, temozolomide, and radiation in newly diagnosed GBM has been launched, and a multicenter, randomized phase III trial in recurrent GBM is underway, comparing cediranib *versus* cediranib plus lomustine *versus* lomustine plus placebo.

The clinical experience with the anti-VEGF pathway agents bevacizumab and cediranib indicates that these drugs may be particularly useful in MG patients for their steroid-sparing effect.[51] In addition, a recent retrospective series suggested that bevacizumab was effective in reducing radiation necrosis.[52] There is increasing awareness that standard treatment with concurrent chemotherapy and radiation may increase the risk of pseudoprogression, tumor necrosis, and edema.[53–55] Further development of antiangiogenic therapy for the treatment of tumor edema and necrosis may be warranted, given that corticosteroids (the most common agents used to treat these indications) are associated with considerable adverse effects.

Vatalanib (PTK787; Novartis, Basel, Switzerland), an inhibitor of the VEGFR1–3, PDGFR, and c-Kit tyrosine kinases, has been clinically investigated in several advanced solid tumors.[56] Several phase I/II clinical trials have studied vatalanib in recurrent GBM patients, either as monotherapy[57] or in combination with either temozolomide or lomustine.[58] Clinical benefits were limited in these studies; response proportions ranged between 4% and 8%, and PFS proportions were not significantly higher than historical benchmarks. These results may have been affected by suboptimal dosing, however. More recently, the combination of vatalanib, the PDGFR inhibitor imatinib (Gleevec; Novartis), and hydroxyurea resulted in a modest response proportion (22%) in a phase I trial of 37 recurrent MG patients.[59]

The broad-spectrum TKIs sorafenib and sunitinib have the capacity to inhibit a number of tyrosine kinases including VEGFR, PDGFR, Flt-3 and c-Kit.[60] Both agents are being studied in early phase clinical trials for recurrent MG. Sorafenib, which can also inhibit Raf, is being evaluated as monotherapy and in combinations with the mammalian target of rapamycin (mTOR) inhibitor temsirolimus (Torisel; Wyeth, Madison, New Jersey) and the epidermal growth factor receptor (EGFR) inhibitor erlotinib (Tarceva; Genentech). Sunitinib is being studied as monotherapy and in combination with irinotecan. Mature data have yet to be reported, although to date these agents appear to be only moderately well tolerated. In addition, several other VEGFR inhibitors are in clinical trials for recurrent MG as single agents or in combination regimens (Table 2).

INHIBITORS OF VEGF-INDEPENDENT ANGIOGENIC SIGNALING PATHWAYS

A number of molecular pathways interact in complex, redundant networks to promote angiogenesis.[8,61] The fi-

Table 2. *Selected Ongoing Clinical Trials of VEGF Pathway Inhibitors*

Agents	Phase	Diagnosis	Sponsor	Primary Endpoint	Sites	Notes
Aflibercept, TMZ, and RT	I	New GBM; recurrent or stable MG	NCI	MTD	NABTC	
Aflibercept	II	Recurrent MG	NCI	PFS6	NABTC	
Bevacizumab and bortezomib	II	Recurrent GBM	Genentech, Millennium	PFS6	Duke	
Bevacizumab and enzastaurin	II	Recurrent MG	NCI	PFS6	NCI	
Bevacizumab and erlotinib	II	Recurrent MG	Genentech	PFS6	Duke	
Bevacizumab and etoposide	II	Recurrent MG	Genentech	PFS6	Duke	
Bevacizumab and LBH589	I/II	Recurrent MG	Novartis Genentech	PFS6	DFCI Northwestern	
Bevacizumab and sorafenib	II	Recurrent GBM	NCI	PFS6	NCCTG	
Bevacizumab and tandutinib	II	Recurrent MG	NCI	PFS6	NCI	
Bevacizumab + RT + TMZ	II	New GBM	Genentech	Survival	UCLA	
Bevacizumab and TMZ	II	New GBM, unresectable or multifocal	Genentech	RR	Duke	≥4 treatment cycles prior to RT
Bevacizumab and TMZ	II	New GBM	Genentech	PFS, RR	Univ. of Chicago	Bevacizumab only after RT
Bevacizumab + RT + TMZ	III	New GBM	Genentech RTOG	PFS, survival	Multiple	
Bevacizumab + RT +TMZ	III	New GBM	Roche	Survival	Multiple	
Bevacizumab and metronomic TMZ	II	Recurrent GBM	Genentech, Schering-Plough	PFS	Duke	
Bevacizumab, TMZ, and erlotinib	II	Stable GBM after RT	NCI	OS, PFS	UCSF	
Bevacizumab and TMZ or etoposide	II	Recurrent GBM after bevacizumab and irinotecan	Genentech	PFS6	Duke	
Bevacizumab, TMZ, irinotecan, and RT	II	New GBM	Genentech, Schering-Plough	OS	Duke	TMZ and bevacizumab throughout; only during irinotecan after RT,
Cediranib or cediranib and lomustine or lomustine	III	Recurrent GBM	AstraZeneca	PFS	Multiple	
Cediranib, TMZ, and RT	I/II	New GBM	NCI	MTD (phase I), PFS (phase II)	MGH, DFCI	
Cediranib, TMZ, and RT	Randomized Phase II	New GBM	NCI, AstraZeneca	PFS and survival	RTOG	
Pazopanib	II	Recurrent GBM	NCI	PFS6	NABTC	
Pazopanib and lapatinib	I/II	Recurrent MG	GlaxoSmithKline	MTD (phase I), PFS6 (phase II)	Multiple	
Sorafenib and bevacizumab	II	Recurrent GBM	NCI	PFS6	NCCTG	
Sorafenib and erlotinib	II	Recurrent GBM	NCI	OS	NABTT	
Sorafenib and erlotinib, tipifarnib, or temsirolimus	I/II	Recurrent GBM	NCI	MTD (phase I), PFS6 (phase II)	NABTC	
Sorafenib and temsirolimus	I/II	Recurrent GBM	NCI	MTD (phase I), PFS6 (phase II)	NCCTG	

(*Table continues*)

Table 2. *Continued*

Agents	Phase	Diagnosis	Sponsor	Primary Endpoint	Sites	Notes
Sorafenib and TMZ	II	Recurrent GBM	Bayer, Schering-Plough	PFS6	Duke	Protracted TMZ schedule
Sorafenib and TMZ	II	New GBM	Bayer	PFS	SCRI	Sorafenib only after RT
Sorafenib, TMZ, and RT	I	CNS tumor	Bayer	MTD	Thomas Jefferson University	
Sunitinib	II	Recurrent MG	NCI	PFS6	Multiple	
Sunitinib and irinotecan	I	Recurrent MG	Pfizer	MTD	Duke	
Vandetanib	I/II	Recurrent glioma	NCI	MTD (phase I), PFS (phase II)	NCI	
Vandetanib, imatinib, and hydroxyurea	I	Recurrent MG	Novartis, AstraZeneca	MTD	Duke	
Vandetanib, TMZ, and RT	I/II	New GBM	AstraZeneca	MTD (phase I), OS (phase II)	DFCI, Multiple	
XL184	II	Recurrent GBM	Exelixis	PFS6	DFCI, MDACC, UCSF, UVa	

DFCI = Dana-Farber Cancer Institute; GBM = glioblastoma multiforme; MDACC = M.D. Anderson Cancer Center; MG = malignant glioma; MGH = Massachusetts General Hospital; MTD = maximum tolerated dose; NABTC = North American Brain Tumor Consortium; NABTT = New Approaches to Brain Tumor Therapy Consortium; NCCTG = North Central Cancer Treatment Group; NCI = U.S. National Cancer Institute; OS = overall survival; PFS6 = 6-month progression-free survival; RR = response rate; RT = radiation therapy; SCRI = Sarah Cannon Research Institute; TMZ = temozolomide; UCSF = University of California, San Francisco; Uva = University of Virginia.

broblast growth factor (FGF) signaling pathway is an important proangiogenic pathway in GBM that is distinct from the angiogenesis driven by VEGF. Recently, the FGF pathway has been implicated in resistance to VEGF-targeted therapy.[18,28,49] Strategies targeting FGF have had limited efficacy in MG patients in earlier trials; however, the inhibitors used in these studies were relatively nonspecific.

Thalidomide, an inhibitor of both basic FGF and VEGF,[62] was shown to have minimal activity as monotherapy or in combinations with carmustine or temozolomide in phase II trials of MG patients.[63–68] The more potent thalidomide analog lenalidomide has better tolerability; however, there was little suggestion of efficacy, either as a single agent[69] or in combination with radiation therapy.[70] These lenalidomide studies were phase I trials and were not designed to assess efficacy, but it is noteworthy that there were no radiographic responses with lenalidomide monotherapy in 24 recurrent GBM patients[69] and one response in 20 evaluable patients with lenalidomide in combination with radiation therapy in newly diagnosed GBM patients.[70] The toxicities associated with this class of agents, which include a potentially overlapping hematologic toxicity between lenalidomide and chemotherapy, may limit further clinical development in MG.

Several other inhibitors of the FGF pathway had limited efficacy in clinical trials of MG patients, including interferon-α,[71] Interferon-β,[72] and suramin.[73,74] However, FGF receptor TKIs with greater specificity for the FGF pathway have been developed; these drugs, which include TKI-258 (Novartis), XL-999 (Exelixis, South San Francisco, California), brivanib (Bristol-Myers Squibb, New York, NY), and BIBF1120 (Boehringer Ingelheim, Germany), have potential utility in MG patients.

The PDGF signaling pathway plays an important role in MG angiogenesis, as well as in glioma transformation[20,75,76], and thus represents a particularly attractive therapeutic target. However, clinical trials evaluating the PDGF receptor inhibitor imatinib as a single agent in recurrent MG patients have been disappointing.[77,78] When imatinib was combined with hydroxyurea, several studies reported promising PFS6 proportions in recurrent MG patients (24%–32%),[79–81] although a recent large multicenter study failed to confirm these findings.[82] Given the abundance of preclinical data implicating the PDGF pathway in glioma angiogenesis and transformation,[20] inhibitors of this pathway continue to be clinically investigated. A study of imatinib, hydroxyurea, and everolimus (RAD001; Novartis), an mTOR inhibitor, in recurrent MG patients is ongoing. The newer PDGF receptor TKIs tandutinib (MLN518; Millennium Phar-

Table 3. *Selected Ongoing Clinical Trials of Non-VEGF Pathway Inhibitors*

Agents	Phase	Diagnosis	Sponsor	Primary Endpoint	Sites
AMG 102	II	Recurrent MG	Amgen	RR	Multiple
Cilengitide	III	New GBM	Merck KGaA	Survival	Multiple
Dasatinib and erlotinib	I	recurrent MG	Bristol-Myers Squibb, Genentech	MTD	Duke
Dasatinib	II	Recurrent GBM	RTOG	RR or PFS6	RTOG
Imatinib, everolimus, and hydroxyurea	I	Recurrent MG	Novartis	MTD	Duke
Imatinib and TMZ	I	Stable or recurrent MG in first relapse	NCI	MTD	Duke
Imatinib, vandetanib, and hydroxyurea	I	Recurrent MG	Novartis, AstraZeneca	MTD	Duke
Tandutinib	I/II	Recurrent GBM	NCI	MTD (phase I), RR (phase II)	NABTT
Tandutinib and bevacizumab	II	Recurrent MG	NCI	PFS6	NCI
TMZ or lomustine + 6-TG, capecitabine, or celecoxib	II	Recurrent MG	MDACC	PFS12	MDACC
TMZ ± thalidomide and/or *cis*-retinoic acid and/or celecoxib	II	Stable GBM after RT	NCI	PFS6	MDACC

GBM = glioblastoma multiforme; MDACC = M.D. Anderson Cancer Center; MG = malignant glioma; MTD = maximum tolerated dose; NABTC = North American Brain Tumor Consortium; NABTT = New Approaches to Brain Tumor Therapy consortium; NCI = U.S. National Cancer Institute; PFS12 = 12-month progression-free survival; PFS6 = 6-month progression-free survival; RR = response rate; RT = radiation therapy; RTOG = Radiation Therapy Oncology Group; TMZ = temozolomide.

maceuticals, Cambridge, MA) and dasatanib (Sprycel; Bristol-Myers Squibb) have potentially greater efficacy in MG patients, due to improved CNS penetration, and are in clinical trials for recurrent MG (Table 3).

Other angiogenic pathways have been targeted in MG patients, although efficacy has been limited in these studies. Signaling through the protein kinase C (PKC) pathway plays an important role in MG angiogenesis. The PKC-β inhibitor enzastaurin (LY317615; Eli Lilly, Indianapolis, IN) was evaluated as monotherapy in a phase III randomized trial, compared against lomustine, in recurrent GBM; however, the study was halted on interim analysis because efficacy milestones were not met.[83] Cyclooxygenase-2 (COX-2), which promotes the expression of proangiogenic factors,[84] has been targeted in recurrent MG patients with the selective COX2 inhibitor celecoxib (Celebrex, Pfizer). However, only modest PFS6 proportions of 19% and 25% were observed when celecoxib was combined with irinotecan and 13-cis-retinoic acid, respectively.[85,86]

The hepatocyte growth factor/scatter factor (HGF/SF) pathway is another important mediator of glioma angiogenesis.[87,88] There has been increased interest in targeting HGF/SF and its cognate tyrosine kinase receptor, c-Met, in MG. Drugs in clinical development for MG that target the c-Met pathway include AMG 102, a fully human monoclonal antibody against HGF/SF, and XL184, a c-Met TKI that also inhibits the VEGFR2 and RET tyrosine kinases.

INHIBITORS OF ENDOTHELIAL CELL MIGRATION

The $\alpha\nu\beta3$ and $\alpha\nu\beta5$ integrins, which are cell surface receptors that promote endothelial cell migration and survival during angiogenesis, represent attractive therapeutic targets.[89] The competitive $\alpha\nu\beta3$ and $\alpha\nu\beta5$ integrin inhibitor cilengitide (EMD121974; Merck, Whitehouse Station, NJ) has demonstrated modest activity in several recent clinical trials of MG patients. In a randomized phase II trial, 81 patients with GBM in first recurrence were treated with cilengitide in one of two dose cohorts. Although the drug was well tolerated, cilengitide exhibited only modest antitumor activity.[90] A radiographic response proportion of 13% and a PFS6 proportion of 15% was observed in the higher dose cohort, with 2000 mg administered twice weekly. A randomized phase II trial reported promising efficacy results using cilengitide in newly diagnosed GBM patients in which 81 patients were randomized to receive either cilengitide in addition to standard therapy (radiotherapy and temozolomide) or standard therapy alone.[91] The primary endpoint was successfully reached; the proportion of patients treated with cilengitide that were progression-free at 6 months (65.4%) was significantly higher than the PFS6 proportion achieved with standard therapy alone (53.6%).[91] Patients with the methylated *O*-6-methylguanine-DNA methyltransferase (*MGMT*) gene promoter appeared to derive the greatest benefit.

Cilengitide toxicity appears to be minimal: no maximum tolerated dose was defined in two phase I studies of cilengitide monotherapy.[92,93] In the randomized trial of cilengitide in newly diagnosed GBM patients, toxicity was similar in both arms of the study.[91] In addition, a recent clinical study observed good tumor penetration after intravenous drug administration.[94] Based on these encouraging results, a multicenter randomized phase III trial is underway using cilengitide in newly diagnosed GBM with methylated *MGMT* gene promoter.

METRONOMIC CHEMOTHERAPY

Conventional chemotherapy administered at low doses on a frequent or continuous schedule, referred to as metronomic chemotherapy, has been shown to target mainly the endothelial cells of growing tumor vasculature.[95] In glioma animal models, metronomic chemotherapy is an effective antiangiogenic strategy.[96] Earlier studies evaluating metronomic chemotherapy in recurrent MG patients reported favorable toxicity profiles, but no significant survival benefits were observed.[97–99] Recently, two reports evaluating low-dosed, frequently administered temozolomide schedules in recurrent GBM patients documented modest gains in PFS6 proportions, relative to historical controls.[100,101] Clinical trials evaluating the addition of bevacizumab to metronomic chemotherapy are ongoing, as this strategy has greater antiangiogenic efficacy in preclinical models.[102] It should be noted, however, that antitumor activity of prolonged temozolomide dosing could also be attributed to the higher cumulative doses and the MGMT enzyme depletion that these schedules achieve.[103]

CURRENT CHALLENGES IN THE CLINICAL DEVELOPMENT OF ANTIANGIOGENIC THERAPY

The compendium of clinical data regarding antiangiogenic agents suggests that these drugs may have modest clinical benefit in a subset of MG patients. This benefit is reflected in an apparent antiedema and steroid-sparing effect, improvements in radiographic response proportions, and a prolonged PFS. Bevacizumab and irinotecan combination therapy is thus frequently used for patients with progressive MG. Nonetheless, a number of issues remain unresolved concerning the clinical use of these drugs, including issues of resistance, radiographic assessment, biomarkers, and toxicity profiles.

Resistance

Clinical data suggest that benefits gained from antiangiogenic agents will be short-lived, with any potential improvements in survival likely measurable in months. Almost all MG patients treated with antiangiogenic inhibitors progress during therapy, as tumors eventually acquire an evasive resistance. After treatment with bevacizumab and chemotherapy fails in MG patients, tumor progression is usually rapid. In a recent series, MG patients treated with a second bevacizumab-containing regimen after progression during treatment with a first bevacizumab regimen had a median PFS of only 37.5 days.[104]

Several mechanisms of resistance to angiogenesis inhibitors have been proposed in GBM.[28,61] Tumors may induce revascularization by activating alternate proangiogenic signaling pathways in response to VEGF inhibition.[28,49,61,105] In recurrent GBM, patients who progressed during cediranib treatment had elevated levels of circulating bFGF, SDF1α, and Tie-2, factors that are mediators of alternative proangiogenic pathways.[49]

A second mechanism of resistance involves the recruitment of vascular progenitor cells and proangiogenic monocytes from the bone marrow.[106] Hypoxia, by inducing hypoxia-inducible factor 1α and its targets SDF1α and VEGF, attracts a heterogeneous population of bone-marrow-derived cells. Endothelial and pericyte progenitors are recruited to the tumor and are directly incorporated as components of new blood vessels, while specific monocytes that are recruited produce proangiogenic factors that facilitate neovascularization.[107,108]

It is increasingly recognized that anti-VEGF therapy may elicit MG tumors to adopt a more invasive phenotype, one that is less dependent on angiogenesis. A number of years ago, experiments in intracranial glioma mouse models demonstrated that VEGF inhibition with antibodies directed against either VEGF or VEGFR generated more invasive tumors that continued to grow by co-opting pre-existing blood vessels.[109,110] These findings were reproduced in recent preclinical experiments using small molecule inhibitors of VEGFR and tumors lacking VEGF.[111] Together, these studies suggest that anti-VEGF therapy may be changing the natural history of tumors by inducing heightened tumor cell invasiveness and, in solid tumor models, enhancing distant metastasis.[111,112] In GBM patients, enhanced infiltration by MRI characteristics have been reported in subsets of patients treated with bevacizumab.[39,44] In these patients, the infiltrating tumor appeared as areas of noncontrast-enhancing increased T_2 or fluid attenuated inversion recovery (FLAIR) signal.

In addition to patients who become resistant after initially responding to anti-VEGF agents, a subset of patients may be intrinsically resistant to therapy.[28,49] Evidence in late-stage breast cancer patients suggests that nonresponding patients may have pre-existing activation of parallel VEGF-independent angiogenic pathways.[113] A recent analysis of tissue obtained from newly diagnosed MG patients suggested that VEGF-independent tumor edema may be mediated by neuronal pentraxin 2

and aquaporin-3.[114] An increasing number of clinical trials are simultaneously targeting multiple proangiogenic pathways to try to pre-empt intrinsic resistance (Table 2).

Lastly, a recent preclinical study demonstrated that survival improvements in mice treated with an anti-VEGF agent were primarily due to alleviation of edema.[115] Animals treated with cediranib had increased survival and decreased tumor-associated edema, but there was no inhibition of tumor growth, and their intracranial glioma xenografts continued to enlarge during treatment. Similar effects on survival, edema, and tumor growth were seen in mice treated with dexamethasone alone. This study raises the possibility that tumors may not develop resistance *per se.* Treatment failure may be due to the inability of anti-VEGF drugs to control cerebral edema and continued enlargement of the tumor rather than to tumor evasive mechanisms. It remains to be determined, however, whether these preclinical studies generalize to other anti-VEGF compounds and to human tumors.

Radiographic assessment of tumor response and progression

Many studies have reported high radiographic response proportions in MG patients treated with antiangiogenic agents. It is becoming apparent, however, that obtaining accurate assessments of response to antiangiogenic therapy is problematic in brain tumor patients.[30] Standard response criteria currently in use for assessing treatment response in brain tumors are dependent on contrast-enhancement on CT or MRI.[29] However, contrast-enhancement on CT and MRI scans reflects VEGF-mediated blood–brain barrier dysfunction and may not be an authentic representation of the underlying tumor. Moreover, anti-VEGF agents decrease permeability of cerebral vessels and diminish the contrast enhancement on standard MRI,[49] making it difficult to distinguish antitumor effects of treatment from the effects of these drugs on blood vessel permeability.

Establishing a reliable measure of treatment response and progression in GBM patients treated with antiangiogenic agents is therefore of paramount importance. An international Response Assessment in Neuro-Oncology working group is currently updating the standard response criteria to address these challenges. Newer imaging techniques that provide functional information may have greater reliability in assessing tumor activity during antiangiogenic treatment. Dynamic contrast-enhanced MRI (DCE-MRI), diffusion-weighted MRI, positron emission tomography (PET), and MRI–PET fusion techniques have potential utility in this setting.[36,116–119] These techniques, however, await validation. A small prospective study reported that [^{18}F]fluorothymidine (FLT) PET response at 1 to 2 weeks after initiation of bevacizumab and irinotecan therapy was predictive of OS in recurrent MG patients.[36] This finding requires validation in larger prospective trials.

Biomarkers

Although several imaging modalities have potential utility in assessing response or progression, there is a need for validated circulating or tissue biomarkers that can accurately predict antiangiogenic efficacy and indicate progression during treatment. Several blood and tissue components have been identified as candidate biomarkers.[27] A recent study suggests that VEGF protein expression in tumor tissue may be predictive for radiographic response in patients treated with bevacizumab.[120] This same study reported tumor expression of hypoxia-induced carbonic anhydrase 9 (CA9) was associated with shorter survival. In recurrent GBM patients, radiographic tumor progression during cediranib treatment was associated with elevated levels of bFGF, SDF1α, Tie-2, and circulating endothelial cells.[49] That study and others have shown that serum VEGF and PlGF are significantly elevated in patients being treated with anti-VEGF therapy.[49,121] These serum markers have potential utility as pharmacodynamic or pharmacokinetic biomarkers during anti-VEGF therapy. Validation of these preliminary findings in large prospective cohorts will be necessary.

Toxicity profile

The risks of hemorrhage and thrombosis have been ongoing concerns with the use of antiangiogenic therapy in MG patients. Cumulative toxicity data indicate that major systemic bleeding is rare, but the risk of epistaxis is increased. The intracranial hemorrhage risk appears to be low, and events are often asymptomatic. Intriguingly, one study treated 11 patients with concurrent antiangiogenic and anticoagulation therapies, and only one episode of mild epistaxis was observed.[39] Although some reports suggest an increased risk of thromboembolic events in MG patients treated with bevacizumab,[32] this risk has been difficult to assess, because patients with GBM have an intrinsically higher risk of thrombosis.[122]

It appears that some toxicities are shared by all inhibitors of angiogenesis, but certain toxicities are associated with specific classes of antiangiogenic agents. Toxicities common to both anti-VEGF antibodies and VEGFR TKIs include fatigue and hypertension. Impaired wound healing is observed, and may be problematic in treating newly diagnosed GBM patients immediately after surgery.[123] Hemorrhage and GI perforation are less frequently observed. With TKIs, skin toxicity, hypertension, diarrhea, and mucositis are more commonly observed, whereas proteinuria is more frequent with bevacizumab. Other rare but serious complications noted with antiangiogenic therapy include myocardial infarction, arterial stroke, reversible posterior leukoencepha-

lopathy syndrome, and thrombotic thrombocytopenic purpura.[33,124,125]

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, current evidence suggests that angiogenesis inhibitors may have clinical utility for MG patients. Clinical benefits have manifested primarily as steroid-sparing effects and increased progression-free survival. Nonetheless, a definite survival advantage has yet to be established with these drugs. Several issues and obstacles remain in the clinical development of antiangiogenic agents in MG, and their resolution may result in improvements in efficacy.

In most MG patients, tumors ultimately become resistant to therapy, often characterized by an increased invasiveness that is difficult to assess and difficult to treat. Because of to the antipermeability effects of VEGF inhibitors, radiographic assessments of response and progression are challenging. Validated biomarkers that predict clinical efficacy, monitor tumor response, and indicate tumor progression do not yet exist. Furthermore, angiogenesis inhibitors possess a unique set of toxicities that are still being characterized in MG patients. Nevertheless, the FDA recently approved bevacizumab for use in recurrent GBM patients, based on the clinical benefits observed in two recent phase II trials.[25,42] This approval represents a milestone in the management of GBM patients, in that bevacizumab would be the first targeted agent with clinical efficacy in this population.

Ongoing clinical trials will address several open questions, including the role of antiangiogenic therapy in newly diagnosed patients. Based on encouraging clinical results in recurrent MG patients, many trials are investigating the addition of antiangiogenic drugs to standard first-line therapy for GBM. Bevacizumab,[123,126] cediranib, vandentanib, aflibercept, XL184, and cilengitide are being evaluated in combination with standard therapy in newly diagnosed GBM patients. Other trials are evaluating combinations of antiangiogenic therapy with different cytotoxic agents or targeted agents in recurrent MG patients in attempts to improve upon the efficacy gains already observed.

Going forward, prospective randomized controlled trials that use survival as an endpoint will be required to determine whether antiangiogenic strategies increase survival of MG patients. Furthermore, clinical trials with well-integrated correlative imaging and molecular studies will be critical to overcoming the challenges that remain. Finally, it is apparent that greater understanding of glioma angiogenesis, the most important angiogenic targets, and the mechanisms of treatment resistance are required. Results from further preclinical investigations and integrative clinical studies may lead to new insights that will hopefully result in improved outcomes for patients with these refractory tumors.

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