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The Emerging Role of Anti-Angiogenic Therapy for Malignant Glioma[†]

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Opinion statement

Adults with glioblastoma multiforme (GBM), the most common primary brain tumor, have an unacceptably poor outcome with conventional cytotoxic therapies. Malignant gliomas are remarkably angiogenic, and vascular endothelial growth factor (VEGF) is the dominant pro-angiogenic factor. Recent clinical trials targeting VEGF signaling have achieved unprecedented rates of durable radiographic and clinical response, while also confirming adequate safety among recurrent malignant glioma patients. An array of additional clinical trials evaluating anti-angiogenic strategies are underway for both recurrent and newly diagnosed malignant glioma patients. Promising results of these approaches suggest that the treatment of GBM may represent an emerging paradigm of anti-angiogenic therapy.

Background

Direct targeting of GBM tumors with cytotoxic therapies has achieved modest benefit due to several factors including high rates of de novo and acquired resistance, heterogeneity within and across tumors, and limited delivery. The current standard of care including surgery, radiation plus temozolomide yields median progression-free and overall survivals of under 7 and 15 months, respectively [1]. Furthermore, there is no effective therapy established for recurrent patients [2]. An evolving alternative therapeutic strategy includes agents that attack GBM tumors indirectly by targeting vital components of the supporting extracellular matrix including the neovasculature.

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a required adaptation for all tumors [3]. Tumor angiogenesis is orchestrated by the simultaneous upregulation of multiple promoters including vascular endothelial growth factor (VEGF), acidic and basic fibroblast growth factor (FGF), interleukins-8 and -6, hypoxia-inducible factor 1-alpha (HIF-1a), and the angiopoietins, with the downregulation of

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endogenous angiogenesis inhibitors such as thrombospondins, angiostatin, endostatin, and interferons [4]. VEGF is the dominant pro-angiogenic factor and, hence, is the primary focus of therapeutic anti-angiogenic interventions.

Successful preclinical and clinical development of therapeutic strategies targeting the VEGF axis has rapidly evolved across multiple solid and hematologic neoplasms. Although malignant gliomas have long been recognized as highly angiogenic [5, 6] and preclinical studies have validated targeting VEGF signaling [7–22], clinical translation has lagged due to toxicity concerns, particularly intracranial hemorrhage. Nonetheless, a recent ground-breaking study evaluating bevacizumab plus irinotecan, not only affirmed the safety of this approach, but also reported unprecedented anti-tumor activity [23••, 24••]. Several additional clinical trials are moving forward to build on these initial results that incorporate a variety of anti-angiogenic strategies (Table 1). In this overview, we will examine angiogenesis in malignant gliomas and the clinical development of anti-angiogenic strategies for patients with these tumors.

CNS Tumor Vasculature: Additional Complexity

- Although malignant gliomas are highly angiogenic [5, 6], perfusion can be paradoxically limited. Malignant glioma blood vessels exhibit complex tortuosity [66] with haphazard interconnections including saccular and blind-ended extensions [67–69]. Furthermore, integrity of the blood brain barrier (BBB), a morphologic, physiologic, and functional protectant of the central nervous system (CNS), is variably compromised in malignant glioma. Diffusion through the intact BBB, normally restricted to small (<400 daltons), lipophilic, non-polar compounds [70–72], is markedly enhanced in malignant gliomas due to an incomplete basement membrane, diminished pericyte coverage, fewer tight junctions, enlarged fenestrae, and increased pinocytosis [73–75]. Furthermore, efflux proteins that normally actively extrude otherwise diffusible molecules from the CNS, are inconsistently present [70, 76–81]. The net result is leaky, intermittent, and unstable blood flow which leads to regional hypoxia, acidosis, and markedly increased interstitial pressure [82•, 83]. Spatially, areas of greatest tumor cell density exhibit the highest vessel count, but often exhibit the most dysfunctional blood flow and greatest permeability. In contrast, advancing regions of infiltrating micrometastatic disease that frequently extend several centimeters maintain a more intact BBB and vascular patterns that resemble normal CNS tissue, as well as near-normal interstitial fluid pressure gradients [84].
- Glial tumors exhibit a prototypic "angiogenic switch," in that induction of pro-angiogenic mediators and new blood vessel formation hallmark the transformation from low-grade to high-grade gliomas [85]. Because malignant gliomas are critically dependent on angiogenesis, they have evolved a highly complex, broad, and redundant network of genetic and cellular signaling cues that drive a remarkably prolific, neovascular capability. VEGF, the dominant pro-angiogenic factor, exists as six homologues (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor) and as biologically active glycoprotein fragments generated by either alternative gene splicing or protease cleavage [86]. VEGF is secreted by tumor cells, infiltrating inflammatory cells, and platelets, and can be sequestered in the extracellular matrix [87–92]. VEGF expression is prognostically relevant [93] and typically is most concentrated adjacent to areas of necrosis and hypoxia including pseudopalisading cells at the leading edge [85, 94–100•].

Table 1. Anti-angiogenic agents currently under evaluation in the treatment of malignant glioma patients

Agent	Primary target	Additional targets	Mechanism/Classification	References
ABT-510	CD36 receptor	–	Synthetic peptide thrombospondin inhibitor	[25]
AZD2171 (cediranib)	VEGFR2	PDGFRβ; c-Kit	Tyrosine kinase inhibitor	[26••]
BAY 43-9006 (sorafenib)	BRAF	VEGFR2-3; PDGFRβ; c-Kit; Ras, p38α	Tyrosine kinase inhibitor	–
CEP-7055	VEGFR1-3	–	Tyrosine kinase inhibitor	[14]
CT-322	VEGFR1-3	–	Adnectin-based competitive inhibitor	–
EMD 121974 (cilengitide)	Integrins $\alpha v\beta 3$, $\alpha v\beta 5$	–	RGD-containing synthetic peptide	[27–36]
GW786034 (pazopanib)	VEGFR1-3	PDGFRβ; c-Kit	Tyrosine kinase inhibitor	–
Interferons α and β	bFGF	–	Suppress expression	[37–41]
Metronomic chemotherapy	Endothelial cells	–	Induces apoptosis of endothelial cells	[42–49]
PTK787 (Vatalanib)	VEGFR2	VEGFR1; VEGFR3; PDGFRβ; c-Kit	Tyrosine kinase inhibitor	[17, 50, 51]
RhuMabVEGF (bevacizumab)	VEGF-A	–	Monoclonal antibody	[23••, 24••, 52•–55•]
SU11248 (sunitinib)	VEGFR2	PDGFRβ; FLT3; c-Kit	Tyrosine kinase inhibitor	[7, 19]
Thalidomide	VEGFR; bFGF	–	Suppress expression	[56–62]
VEGF-Trap	VEGFA, B, PIGF	–	Decoy receptor	[63–65•]
ZD6474 (vandetanib)	VEGFR2	EGFR; RET	Tyrosine kinase inhibitor	[9, 18, 20]

c-Kit—a member of the platelet-derived growth factor receptor family; EGFR—epidermal growth factor receptor; FLT-3—FMS-related tyrosine kinase 3; PDGFRβ—platelet-derived growth factor receptor-β; PIGF—placental growth factor; VEGF—vascular endothelial growth factor; VEGFR—vascular endothelial growth factor receptor

- Several hypoxia-dependent and hypoxia-independent mechanisms contribute to abundant VEGF expression in malignant glioma [83, 85, 101–104]. Hypoxia, a common feature of GBM, enhances expression and stabilization of hypoxia inducible factor-1a (HIF-1a), a transcription factor that activates myriad target genes regulating tumor angiogenesis, migration, and survival, including VEGF and VEGF receptors (R) [85, 105–108]. Increased expression and activation of many growth factors, often in parallel with their cognate receptors, are linked with increased VEGF activity in malignant gliomas including epidermal growth factor (EGF)/EGFR [109, 110], platelet-derived growth factor (PDGF)/PDGFR [111–113••], scatter factor/hepatocyte growth factor (SF/HGF)/MET [114], insulin-like growth factor (IGF)/IGFR [115, 116], stem cell factor/c-Kit [117], and fibroblast growth factor (FGF) [98, 118–120]. In addition, the phosphatidylinositol 3-kinase (PI3K/Akt) and the Ras/mitogen-activated protein kinase (MAPK) signaling pathways, which are commonly activated in GBMs, lead to increased VEGF expression [109].
- Expression of VEGFRs (VEGFR-1, VEGFR-2, and VEGFR-3) and co-receptors including the neuropilins, although very low in the normal brain, is also markedly increased in malignant gliomas [94, 96–98, 121, 122]. Activation of VEGFRs by ligand binding triggers an intracellular signaling cascade that promotes endothelial cell proliferation, survival, activation, invasion, migration, and permeability [102, 123]. VEGF signaling also activates endothelial cell nitric oxide synthase to generate nitric oxide [124] and signals bone marrow-derived endothelial cell progenitors to mobilize to distant sites of tumor angiogenesis [125–128].
- Several pro-angiogenic factors in addition to VEGF are also upregulated in malignant gliomas [129] including FGF [130], tumor necrosis factor-α (TNF-α) [131], interleukin-8 (IL-8) [132–134], interleukin-1β, interleukin-6, interferons [135], cyclooxygenase-2 (COX-2) [136], and nitric oxide [137]. Furthermore, increased expression of factors mediating endothelial cell invasion is also common in malignant glioma including matrix metalloproteinases (MMP)-2 and MMP-9, urokinase-type plasminogen activator (uPA) and its receptor (uPAR), cathepsin-B, integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, and tenascin-C [138–143]. Many of these factors have been successfully targeted in preclinical studies [27, 144–149]. Alternatively, endogenous angiogenesis inhibitors, commonly deficient in GBMs, can be augmented to provide therapeutic benefit in preclinical studies [25, 148–152].

Emerging Insights: Potential Mechanisms of Anti-Angiogenic Therapy

- Growing evidence suggests that anti-angiogenic agents may affect tumors at multiple levels. First, by decreasing the blood supply, tumors can be deprived of vital nutrients and oxygen. Second, anti-angiogenic agents may sensitize tumor endothelial cells to cytotoxins [153, 154•]. Third, anti-angiogenic agents can counteract a surge in VEGF and/or accelerated tumor cell repopulation induced by cell killing following cytotoxic therapy [155•, 156].
- A fourth hypothesis contends that anti-angiogenic agents may selectively "prune" tumor vasculature, thereby transiently normalizing perfusion to improve chemotherapy delivery [157, 158•]. In support of this model, lowered interstitial fluid pressure (IFP), higher oxygen content, and decreased permeability were observed in an orthotopic GBM model following anti-angiogenic therapy [159–161]. Furthermore, decreased

perfusion, vascular volume, microvessel density, and IFP, along with increased pericyte coverage, were observed among colorectal cancer patients treated with bevacizumab plus chemotherapy [162••]. Further clinical evidence supporting the vascular normalization hypothesis was recently reported among recurrent GBM patients treated with the pan-VEGFR inhibitor, AZD2171 (cedarinib, AstraZeneca, UK). Using an extensive battery of sophisticated imaging modalities, responding patients also had evidence of decreased tumor vessel size, permeability, blood volume, and blood flow [26••].

- Fifth, anti-angiogenic agents have also recently been shown to target cancer stem cells. Brain tumor stem cells, identified by CD133 (prominin-1) cell surface expression, exhibit marked proliferative capacity, extensive self-renewal, diverse differentiation capability, and the ability to recapitulate complex tumors following xenotransplantation [163–165]. Recent studies demonstrate that GBM stem cells generate highly angiogenic and aggressive tumors upon xenotransplantation due to substantially upregulated VEGF expression compared to minimally active and essentially non-angiogenic tumors derived from CD-133 negative cells [166••]. Additional studies suggest that the self-renewal and tumor-forming abilities of CD-133 positive cells are critically dependent on a bi-dimensional interaction with endothelial cells within the immediate microenvironment, referred to as the perivascular niche [167••]. Of note, anti-angiogenic therapy, including bevacizumab and metronomic chemotherapy, suppresses GBM stem cell tumorigenicity [166••–168•]. The cumulative findings to date suggest that effective anti-angiogenic therapy may target GBM stem cells directly and may also critically perturb the perivascular niche required for stem cell well-being [169•].

Clinical Studies: Targeting VEGF

Bevacizumab

- Based on phase III trials demonstrating survival improvements for patients with colorectal, breast, lung, and pancreatic cancer treated with bevacizumab (BV) plus cytotoxic chemotherapy [170], an initial study of BV plus the topoisomerase-1 inhibitor irinotecan among patients with recurrent malignant glioma (Camptosar, Pfizer, New York, USA) was performed [23••, 24••, 171]. The primary endpoint was 6-month PFS. Irinotecan was included in the study regimen because it has modest activity among recurrent malignant glioma patients including a radiographic response rate of 5–15% and a median PFS of approximately 12 weeks [172–176].
- Adult patients with measurable, recurrent grade 3 or 4 malignant glioma with up to three prior relapses were eligible. Patients were required to have a Karnofsky performance status of ≥60%, adequate bone marrow, hepatic and renal function, no evidence of blood on pretreatment imaging, and be at least six weeks from prior surgery and four weeks from prior radiation therapy or chemotherapy (6 weeks for nitrosoureas). Prior BV treatment or current warfarin administration excluded patients.
- A total of 68 patients enrolled including an initial cohort of 32 patients treated with BV (10 m/kg) and irinotecan every two weeks, followed by a second cohort of 36 patients treated with BV every three weeks (15 mg/kg) and irinotecan on weeks 1, 2, 4, and 5 (Fig. 1). The irinotecan dose for patients on CYP-3A enzyme-inducing

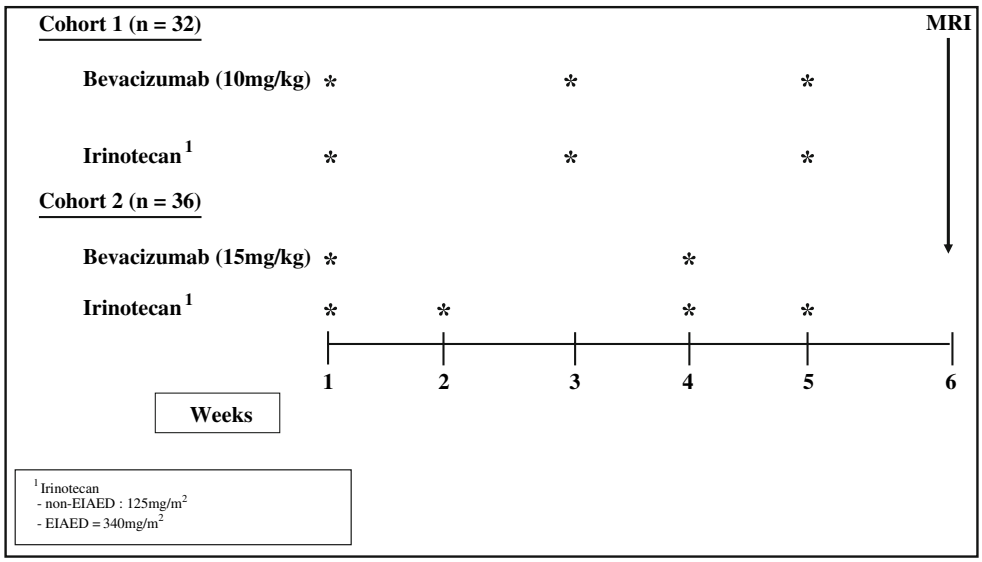

Figure 1. Treatment schema for recurrent malignant glioma patients treated with bevacizumab plus irinotecan [23••, 24••]

anti-epileptic drugs (EIAEDs), including phenytoin, carbamazepine, oxcarbazepine, phenobarbitol, and primidone, was 340 mg/m^2 while those not on EIAEDs received 125 mg/m^2 [177]. Patients were evaluated with a complete physical examination and MRI after each 6-week cycle. MacDonald criteria [178] were used to classify response. In addition, radiographic response also required stable or improved T2, and fluid attenuated inversion recovery (FLAIR) signal abnormalities. Patients with clinical decline felt to be due to underlying tumor, regardless of imaging findings, were classified as progressive.

- Patient characteristics for both cohorts are summarized in Table 2. Enrolled patients had a median of two prior episodes of progressive disease and all had received prior temozolomide-based chemoradiation.
- Overall, toxicity was acceptable. Two CNS hemorrhages occurred (3%) including a patient in cohort one after 10 cycles of therapy, and a patient on enoxaparin in cohort two after 9 cycles. Four patients from each cohort (12%) developed thromboses, including one patient with an arterial cerebrovascular stroke. Therapy was discontinued in four patients (6%) due to fatigue or gastrointestinal toxicity, and in two patients due to grade 2 proteinuria.

Table 2. Characteristics of patients treated with bevacizumab plus irinotecan [23••, 24••]

Characteristics	Cohort 1	Cohort 2
Number of patients	32	36
Male:Female	21:11	24:12
Median age (years; range)	49 (27–66)	46 (18–62)
Karnofsky Performance Status	80 (60–100)	80 (60–100)
Grade IV:Grade III	23:9	12:24
Median number of progressions (range)	2 (1–3)	2 (1–3)
Median time from diagnosis (months; range)	14 (3–66)	42 (3–165)
Anticonvulsant EIAED:non-EIAED	14:18	17:19

- The rate of radiographic response in comparison to historical benchmarks is summarized in Table 3. Of note, despite being more heavily pretreated and having failed prior temozolomide, patients treated with BV plus irinotecan had much higher rates of radiographic response compared to patients treated with temozolomide [179, 180]. The rates of radiographic response and stable disease did not differ between patients on cohorts 1 and 2. Figure 2 demonstrates representative MRI images of response to bevacizumab plus irinotecan.
- Salvage therapies for GBM patients historically achieve single-digit response rates with most patients progressing initially (Table 3) [2, 180, 181]. In contrast, the ratio of PD to response for patients treated with BV plus irinotecan was reversed. Specifically, the majority of patients responded while only a single-digit rate of progressive disease (6%) was observed. Finally and most encouragingly, clinical and neurologic status commonly reflected radiographic findings in that most patients with a radiographic response also improved neurologically, with most also able to taper or discontinue chronic dexamethasone dosing. Of note, similar rates of radiographic response to BV plus chemotherapy were recently described in two, smaller, additional reports [52•, 53•].
- Furthermore, responses to BV and irinotecan were durable (Table 3). The median PFS for grade 3 and 4 patients was nearly two-fold greater than that achieved with temozolomide at first recurrence, and the 6-month PFS rate was greater [179, 180]. Of note, six patients with recurrent GBM (18%) completed a year of therapy, including five patients with no hypermetabolic activity on FDG-PET imaging at therapy completion, suggesting the absence of residual active tumor. Similarly, 7 patients with recurrent grade 3 tumors (21%) completed one year of therapy including six with negative FDG-PET scans at study completion.
- In addition, median overall survival for patients treated with BV plus irinotecan significantly surpassed that reported in the literature (Table 3) [179–181]. While it is possible that decreased permeability and lowered contrast uptake induced by BV may have contributed to the radiographic response rate, the considerably improved rates of PFS and OS observed with BV plus irinotecan therapy strongly support an underlying anti-tumor action of this regimen.
- A randomized study comparing GBM patients at first or second recurrence to either BV alone or the combination of BV plus irinotecan has recently been performed to further assess the anti-tumor activity of these agents. However, in a recent report of 15 recurrent malignant glioma patients treated with 15 mg/kg of BV alone every 3 weeks, only 2 patients achieved a PR (13%), while 5 were stable (33%) and 8 progressed (53%) [54]. Although these results are preliminary, they suggest an inferior disease control rate by BV alone compared to that achieved with BV plus irinotecan.
- As stated above, many patients with radiographic response reported by Vredenburgh *et al.* also improved neurologically [23••, 24••]. Similarly, parallel radiographic and clinical improvement, as reflected in the required daily dose of dexamethasone, was observed among eight patients with CNS tumors and radiation necrosis treated with BV. Of note, seven of the eight patients also received chemotherapy with BV. Specifically, a 48% (22% SD) average decrease in T1-weighted post-Gd-contrast measurements and a 60% (18% SD) decrease in FLAIR changes were reported, while all patients on pre-treatment dexamethasone therapy substantially reduced dexamethasone by an average of 8.6 mg (3.6 mg) per day [55•].

Table 3. Outcome achieved with bevacizumab plus irinotecan among patients with recurrent grade 3 and grade 4 malignant glioma compared to patients enrolled on prior salvage studies

	Grade 3			Grade 4		
Outcome	**BV + CPT-11 [23••, 24••] (*n* = 34)**	**TMZ 1st PD [178] (*n* = 162)**	**Other Salvage Therapy [179] (*n* = 150)**	**BV + CPT-11 [23••, 24••] (*n* = 34)**	**TMZ 1st PD [180] (*n* = 112)**	**Other salvage therapy [179] (*n* = 225)**
CR/PR (%)	65	35	14	53	5	6
SD (%)	32	27	34	41	40	27
PD (%)	3	38	52	6	55	67
PFS (median, weeks)	42	22	13	23	12	9
6 month PFS (%)	61	46	31	43	21	15
Overall survival (median, weeks)	60	54	47	40	30	25

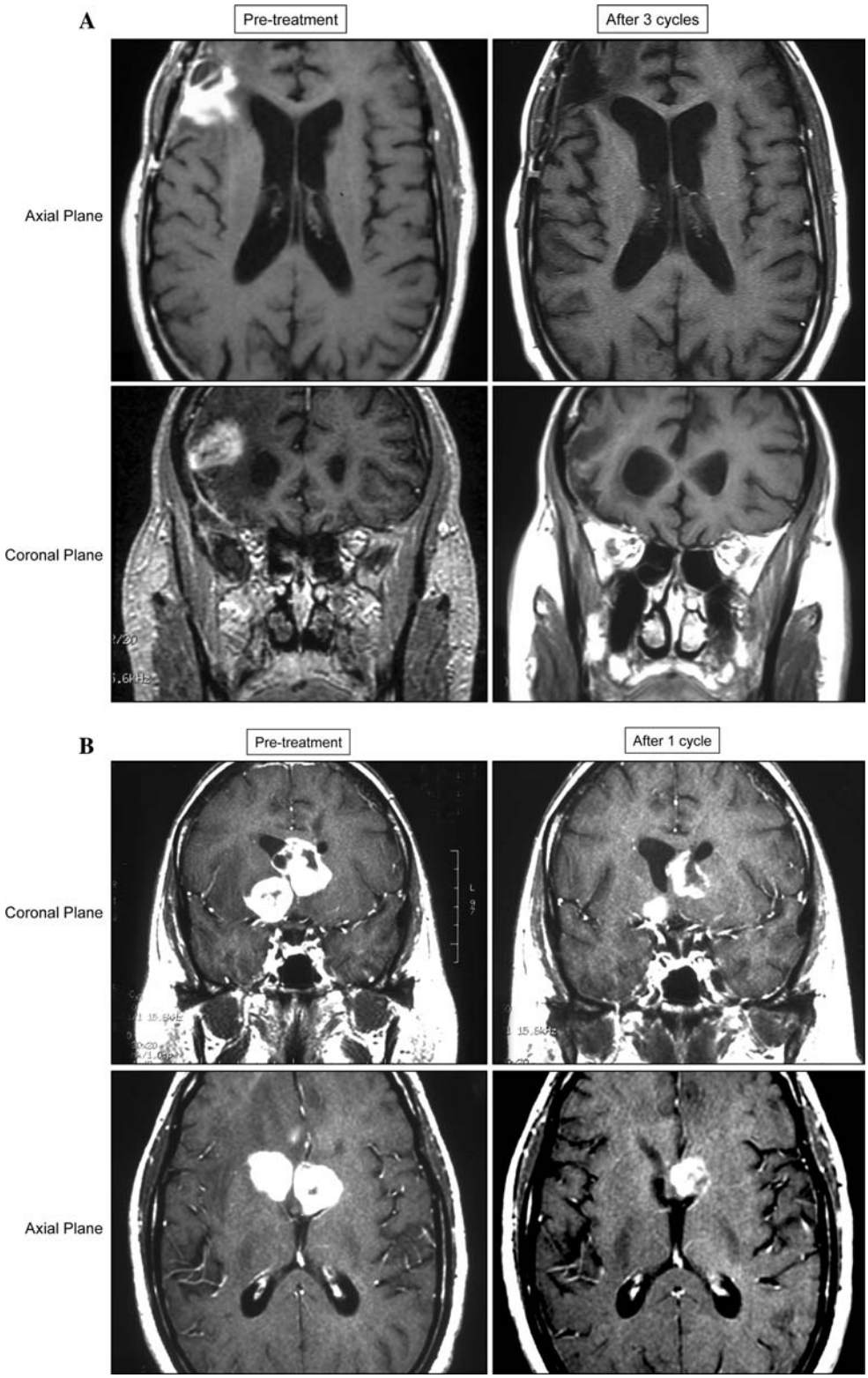

Figure 2. T-1 weighted MRI scans following gadolinium administration demonstrating representative complete (panel a) and partial (panel b) radiographic responses of recurrent GBM patients following treatment with bevacizumab plus irinotecan

- Multiple additional studies are ongoing to evaluate alternative BV-based regimens for recurrent malignant glioma patients. Two separate single-arm studies combining BV with protracted, metronomic dosing schedules of either temozolomide or etoposide are underway, while the Radiation Therapy Oncology Group (RTOG) is randomizing recurrent GBM patients to receive BV with either protracted temozolomide (75 mg/m^2/day for 21 days each month) or irinotecan every 2 weeks. An additional study combining BV plus daily erlotinib is also underway.
- Preclinical studies confirm that inhibition of VEGFR signaling potentiates radiotherapy in GBM models [182, 183]. Therefore, several single institutional studies are underway to evaluate the addition of BV to temozolomide chemoradiation for newly diagnosed GBM patients. In addition, a multi-center, randomized phase III clinical trial for newly diagnosed GBM patients is being planned.

VEGF receptor tyrosine kinase inhibitors

- Receptor tyrosine kinase (RTK) inhibitors are typically small molecules that competitively block tyrosine or serine/threonine kinase domains located intracellularly. Preliminary results of VEGF RTK inhibitors under evaluation for malignant glioma patients have been reported. Nine of 16 patients (56%) treated with cediranib (AZD2171, AstraZeneca, UK), a potent, oral, pan-VEGFR, PDGFR, and c-kit inhibitor, achieved a radiographic response, while 3 additional patients achieved stable disease. In addition, 8 of 11 patients (73%) were able to reduce pretreatment corticosteroid dosing. The median time to progression was 15.8 weeks. Elegant collaborative imaging studies revealed that decreased contrast enhancement was accompanied by significant decreases in tumor vessel size, permeability, blood volume, and blood flow, consistent with "normalization" of tumor vessels. Of note, reversal of tumor vessel normalization was observed following drug interruption [26••]. A multi-center, randomized clinical trial is planned to evaluate cediranib versus lomustine versus the combination of cediranib plus lomustine in patients with recurrent GBM.
- Vatalanib (PTK787/ZK222584; Novartis, NJ, USA), a potent inhibitor of VEGFR1–3, has been evaluated following single-agent administration as well as in combination with either temozolomide or lomustine chemotherapy. Only modest rates of radiographic response and progression-free survival were reported which may have been affected by suboptimal dosing [50, 51]. A phase I study of vatalanib plus the PDGFR inhibitor, imatinib mesylate, for recurrent malignant glioma patients has recently completed, and a clinical trial of Vatalanib plus radiotherapy and temozolomide for newly diagnosed GBM patients is ongoing.
- Several additional RTK inhibitors targeting VEGF are currently being evaluated in ongoing clinical trials for malignant gliomas (Table 1).

Decoy ligand: VEGF-TRAP

- VEGF-TRAP (Regeneron, NY, USA) acts as a soluble decoy VEGF receptor that binds circulating VEGF thereby preventing it from interacting with its receptors on tumor endothelial cells [63, 64]. In preclinical GBM xenografts,VEGF-TRAP potentiates radiotherapy

[65•]. The North American Brain Tumor Consortium recently initiated a single-arm phase II trial of VEGF-TRAP monotherapy among patients with recurrent malignant glioma following temozolomide failure, while a multi-center clinical trial incorporating VEGF-TRAP with temozolomide chemoradiotherapy is planned for newly diagnosed GBM patients.

Clinical Studies: Targeting Other Angiogenic Mediators

FGF inhibitors

- Although thalidomide can inhibit both bFGF and VEGF angiogenic signaling [56], only modest anti-tumor activity was observed among recurrent patients treated with either single-agent thalidomide or thalidomide plus carmustine [57–60]. Disappointing results were also reported among newly diagnosed GBM patients treated with thalidomide plus temozolomide [61, 62]. Additional studies with thalidomide are unlikely based on the lack of an optimal biologic dose and its association with several limiting toxicities including fatigue, polyneuropathy, and thromboses. Interferons-α and -β can also block FGF-mediated angiogenesis [37]. Unfortunately, these agents also exhibit limited efficacy and frequent toxicity [38–41].
- FGFR TKIs are under development including TKI-258 (Novartis International AG, Basel, Switzerland) and XL-999 (Exelexis Inc., South San Francisco, CA, USA). These agents may provide improved efficacy, yet incur less toxicity due to enhanced specificity. Furthermore, lower toxicity profiles may facilitate combination with complementary anti-angiogenic agents.

Metronomic Chemotherapy

- Low-dose, protracted administration schedules of conventional cytotoxic chemotherapeutic agents, also referred to as metronomic chemotherapy, inhibit angiogenesis by impairing endothelial cell proliferation and survival [42]. Preclinical studies demonstrate activity of this approach in malignant glioma models [43–45]. Although well-tolerated, limited anti-tumor benefit has been observed to date among recurrent malignant glioma patients [46]. However, initial reports suggest that regimens combining metronomic chemotherapy with complementary anti-angiogenic agents may improve efficacy [47–49]. Additional clinical trials combining metronomic chemotherapy with more potent anti-angiogenic agents, such as bevacizumab, are ongoing.

Anti-integrin therapy

- Integrins, cell surface receptors widely expressed on GBM cells and tumor endothelium, interact with multiple extracellular ligands, including vitronectin, fibronectin, laminin, fibroblast-growth factor, MMP-2, thrombospondin, fibrin, and fibrinogen, via an arginine-glycine-aspartic acid (RGD) peptide sequence to modulate intracellular signal transduction and promote tumor cell invasion, migration, proliferation, survival, and angiogenesis [142, 184–186].
- Integrin inhibitors including cilengitide (EMD 121974, Merck KgaA, Darmstadt, Germany), a cyclic RGD peptide that competitively binds $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin receptors [27–29], are active in preclinical

GBM models [30–32]. Clinical activity was reported in a recent phase I study among recurrent GBM patients treated with single-agent cilengitide including objective radiographic responses in 5 of 51 patients (10%) and stable disease in 16 patients (31%) for a median of 5.4 months across a wide range of cilengitide dose levels [33•]. Cilengitide is very well tolerated with no dose-limiting toxicities occurring in two separate phase I studies despite dose levels up to 2400 mg/m^2 twice weekly [33•, 34]. Additional anti-tumor benefit was recently reported in a phase II study that randomized recurrent GBM patients to either an intermediate-low (500 mg) dose or an intermediate-high (2000 mg) dose. In this study, no reproducible toxicities were observed on either arm, and outcome trended more favorably among patients treated at the higher dose level, including a 6-month PFS of 15% [35]. An additional trial evaluating intratumoral pharmacodynamics and pharmacokinetics of cilengitide is ongoing by the NABTC among recurrent GBM patients treated with cilengitide prior to scheduled, debulking surgery. Furthermore, preliminary results of a trial with cilengitide (500 mg twice weekly) plus temozolomide chemoradiotherapy among newly diagnosed GBM patients recently reported encouraging 6-month PFS and OS at 12 months, and confirmed safety of this approach [36]. A similar single-arm trial evaluating cilengitide dosed at 2000 mg in combination with temozolomide chemoradiotherapy is being conducted by the New Approaches to Brain Tumor Therapy (NABTT) cooperative group among newly diagnosed GBM patients. Finally, a multi-center, randomized phase 3 study for newly diagnosed GBM patients is planned.

Evolving Challenges

Assessing activity

- Increasing controversy questions the reliability of traditional assessment of malignant glioma based on contrast-enhanced MRI. For example, the phenomenon of "pseudo-progression" is increasingly recognized following conventional temozolomide chemoradiation for newly diagnosed GBM patients [187]. Conversely, potent anti-VEGF agents may decrease permeability and lessen contrast enhancement resulting in a potential "pseudo-response." Clarification of this critical issue will require further clinical investigation evaluating additional imaging approaches such as positron emission tomography (PET) [188–190], magnetic resonance spectroscopy [191], and complementary MRI techniques, such as dynamic contrast-enhanced MRI [192–194], dynamic susceptibility MRI [195–197], arterial spin labeling [198], and high-resolution magnetic resonance angiography [199, 200].

Toxicity

- The distinct toxicities of anti-angiogenic therapeutics present additional challenges for neuro-oncology patients [201]. Fatigue, although nearly universal in frequency, is tolerable in a majority of cases. Similarly, hypertension can be managed in most cases. An increased risk of thromboses [202], including arterial events, as well as impaired wound healing, is also a well-described sequela of these agents and must be carefully considered among treated patients, particularly

when administered within several weeks of surgery. Although experience to date indicates a low risk of intracranial hemorrhage, clinicians must maintain vigilant awareness, and patients should avoid exposure to additional risk factors for bleeding. Intestinal perforation has also been observed among recurrent malignant glioma patients, particularly those with pre-existing intestinal polyps. Nasal septal perforation has also been observed. Reversible posterior leukoencephalopathy (RPLS), a syndrome of acute cortical blindness and hypertension with subcortical white matter T2 and FLAIR changes, has been reported following BV therapy [203, 204]. Fortunately, most cases resolve with stringent blood pressure control. Finally, toxicities associated with long-term use of anti-angiogenic agents remain poorly defined.

Biomarkers

- The identification and validation of biomarkers of activity will greatly enhance optimal integration of anti-angiogenic therapeutics into successful treatment strategies for neuro-oncology patients. For example, biomarkers may better define optimal dosing schedules, more rapidly identify patients likely to benefit (or conversely to fail), and predict the emergence of resistance [205]. Effective biomarkers must be sensitive, specific, cost-effective, efficient, and associated with low rates of interoperator variability. Promising candidate biomarkers include correlative imaging parameters [53•, 206•], as well as circulating factors such as plasma VEGF [26••, 207–211], bFGF [26••, 211], tumor stromal-derived factor-1 (SDF1α) [26••], and viable circulating endothelial cells [26••, 127, 208, 212].

Resistance

- Initially, lack of resistance to anti-angiogenic therapeutic strategies was predicted based on the normal and stable genetic makeup of tumor vasculature relative to the genomic instability and mutation frequency typically present in tumors [213]. However, treatment failure among initially responsive patients suggests that resistance to anti-angiogenic agents is relevant. Several potential mechanisms of resistance have been identified including compensatory upregulation of alternative angiogenic factors such as PDGF/PDGFR-β and Ang-1/Tie-2 following VEGF inhibition [214, 215], increased mobilization of pericytes, the secretion of endothelial cell survival factors [214], and the ability of glioma cells to induce a more invasive phenotype, accompanied by normal host blood vessel co-option and eventual gliomatosis [12, 216–219]. Future regimens that target multiple angiogenic mediators as well as regimens that inhibit key mediators of both tumor angiogenesis and invasion may help circumvent some emerging resistance mechanisms [19, 45, 220].

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Papers of particular interest, published recently, has been highlighted as:
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