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ANTI-ANGIOGENIC APPROACHES TO MALIGNANT GLIOMAS

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ABSTRACT

Despite advances in multidisciplinary approaches, the prognosis for most patients with malignant gliomas is poor. Malignant gliomas are highly vascularized tumors with elevated expression of endothelial growth factor (VEGF), an important mediator of angiogenesis. Recent studies of bevacizumab, an anti-VEGF monoclonal antibody, alone or associated with chemotherapy, have demonstrated high response rates and prolongation of median and 6-month progression-free survival. Clinical evaluation of several multitarget small molecule tyrosine kinase inhibitors is ongoing. Other promising antiangiogenic compounds are cilengitide and continuous temozolomide. Toxicity is acceptable. Open issues are represented by patterns of tumor progression, resistance mechanisms and biomarkers.

KEYWORDS

Glioblastoma, VEGF, bevacizumab, cilengitide, temozolomide, toxicity, resistance.

INTRODUCTION

Malignant gliomas are the most common malignant primary brain tumors. Currently, standard therapy for glioblastoma (WHO grade 4) involves maximal safe surgical resection, followed by radiotherapy with concomitant and adjuvant temozolomide [1, 2]. Despite optimal treatment, glioblastomas inevitably recur with a median survival of about 15 months. For anaplastic gliomas (WHO grade 3) surgery, radiotherapy and chemotherapy results in a median survival of approximately 36 months [3]. Treatment options for recurrent malignant gliomas are limited, there is no widely accepted standard of care and the prognosis is poor. In the large database of Wong et al [4], that included 225 patients with recurrent glioblastoma enrolled in 8 consecutive phase II chemotherapy trials, the 6-month progression-free survival was 15% and the median overall survival was 25 weeks. The formation of blood vessels occurs throughout embryonic and adult life. In the embryo, a primitive vascular plexus is formed *de novo* by the aggregation of endothelial cell (EC) precursors known as angioblasts [5]. This process, termed vasculogenesis, forms the early blood vessels that supply developing organs with nutrients and trophic signals. A complex process of vessel sprouting, growth and remodeling then results in the development of a functional circulatory system [6]. This formation of new blood vessels from pre-existing vasculature, known as angiogenesis, also contributes to organ growth after birth. In adulthood, angiogenesis occurs only in the cycling ovary and in the placenta during pregnancy. However, angiogenesis is reactivated in wound healing, and in response to physiological stimuli such as hypoxia and inflammation [7]. Angiogenesis is regulated by numerous stimulatory (pro-angiogenic) and inhibitory (anti-angiogenic) factors that exist in a finely tuned balance.

Angiogenesis is one of the hallmarks of the malignancy [8]. For tumors to grow beyond 2 mm^3, new vasculature is required for adequate nutrient delivery and waste removal [9]. The induction of tumor vasculature, termed the "angiogenic switch", is a required discrete step of malignant transformation and

results in exponential growth of the tumor beyond the limit of a few mm [10] . In 1971, Judah Folkman hypothesized that inhibiting angiogenesis would be an effective antitumor strategy [11]. Since then, the understanding of the biology of angiogenesis has dramatically increased. Many regulators of angiogenesis have been identified, of which vascular endothelial growth factor (VEGF) is the most extensively studied [7].

ANGIOGENESIS AND MALIGNANT GLIOMAS

Malignant gliomas have the highest degree of vascular proliferation among solid tumors [12], and angiogenesis is a crucial step in the development and progression [13]. Increased microvessel density in glial tumors is correlated with degree of malignancy, biological aggressiveness, clinical recurrence, and poorer survival [14]. Thus, inhibition of angiogenesis appears to be an attractive strategy for treatment.

Angiogenesis involves a well-characterized cascade of events governed by numerous mediators. Initially, VEGF increases vascular permeability, and then pericyte coverage of vessels is reduced and the basement membrane (BM) and extracellular matrix (ECM) are degraded. VEGF and other factors then stimulate EC proliferation, migration, and tubule formation. Finally, new BM and ECM are formed and pericytes are recruited to the new vessels [7]. Gliomas may initially grow in an angiogenesis-independent manner by co-opting pre-existing host vessels [15]. Glioma cells first accumulate around existing vasculature and mechanically disrupt contacts between ECs and pericytes. ECs of the co-opted vessels respond by expressing angiopoietin-2 (Ang-2), leading to vessel destabilization, decreased pericyte coverage, and vessel regression [16]. The area supplied by the involuted vessels becomes hypoxic and necrotic, with pseudopalisades of neoplastic cells surviving in a rim around the necrotic area. Hypoxia then induces expression of hypoxia inducible factor-1a (HIF-1a) and VEGF in the tumor cells, triggering angiogenesis adjacent to the necrotic area [17]. Molecular alterations in tumor cells, such as epidermal growth factor receptor (EGFR) overexpression, and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) loss also increase VEGF expression and promote angiogenesis [18]. The resultant tumor vasculature is highly distorted and functionally abnormal, with increased vascular permeability, irregular blood flow, and hemorrhages [19]. In glioblastomas, vascular proliferation takes the form of characteristic “glomeruloid” microvascular tufts [20] that consist of hyperplastic ECs surrounded by an irregular BM and a discontinuous layer of pericytes and vascular smooth muscle cells [21].

Hypoxia is a potent stimulator of angiogenesis in malignant gliomas, being the HIF-1 transcription factor the critical mediator of hypoxia-induced angiogenesis. Other factors, that affect glioma angiogenesis, include fibroblast growth factor (FGF) [22], platelet-derived growth factor (PDGF) [18], hepatocyte growth factor/scatter factor (HGF/SF) [23], angiopoietins, interleukin (IL)-6 and IL-8 [24], angiostatin, endostatin, and thrombospondins [25]. Moreover, increased signaling through a number of growth factor receptors, such as insulin-growth factor receptor (IGFR), stem cell factor receptor (c-Kit), and fibroblast growth factor receptor (FGFR), has also been shown to enhance VEGF activity [26].

Several VEGF family members and biologically active splice variants have been described so far, including VEGF-A to D and placental growth factor. These act through receptor tyrosine kinases, among which at least three receptor subtypes have been identified (VEGFR-1, VEGFR-2, and VEGFR-3). Both endothelial cells and glioma cells themselves may express and upregulate VEGF and its receptors, resulting in both paracrine and autocrine loops that drive endothelial cell proliferation, invasion, migration, and permeability. The level of VEGF expression has been shown to correlate with the degree of malignancy and tumor prognosis [14, 23].

Cancer stem cells appear to be directly involved in stimulating tumor angiogenesis through production of proangiogenic molecules such as VEGF [27]. Similar to neural stem cells that reside in neurovascular niches, cancer stem cells are thought to persist in close proximity to the tumor vasculature [28, 29]. Thus, inhibition

of tumor angiogenesis may also potentially target the "Achilles heel" of the tumor itself, the cancer stem cells, with the hope of achieving more durable clinical responses.

ANTIANGIOGENIC STRATEGIES IN MALIGNANT GLIOMAS

Anti-angiogenic strategies have a few theoretical advantages over conventional chemotherapy or direct tumor cell targeting approaches. Anti-angiogenic agents primarily target ECs, which are in direct contact with the blood, and therefore blood-brain barrier concerns may be less important. Because angiogenesis is absent in adults aside from a few physiologic processes, anti-angiogenic therapy is presumably highly tumor-specific and associated with low toxicity. ECs are genetically more stable than tumor cells and therefore theoretically less likely to develop resistance to therapy. A large body of preclinical evidence has validated the anti-tumor efficacy of angiogenesis inhibition [30, 31]. A number of anti-angiogenic molecularly targeted agents have been developed. In malignant gliomas, the most clinically advanced strategies are mAbs directed against growth factors or their receptors, receptor TKIs, and molecules that block ligand-receptor interactions.

Targeting Angiogenesis through VEGF Inhibition

The clinical breakthrough for anti-angiogenic therapy came when a phase III trial demonstrated a modest but significant increase in overall survival (OS) following the addition of bevacizumab to chemotherapy (irinotecan/ 5-flourouracil/ leucovorin) in previously untreated metastatic colorectal cancer patients [32], leading to the FDA approval of bevacizumab. Subsequent phase III clinical trials in advanced colorectal, breast, and lung cancers demonstrated increased OS or progression-free survival (PFS) when bevacizumab was combined with cytotoxic chemotherapy [33]. In malignant gliomas, VEGF pathway inhibition is the most clinically developed anti-angiogenic strategy.

The first usage of bevacizumab in patients with recurrent malignant gliomas was in an uncontrolled clinical trial by Stark-Vance et al in 2005 [34]. A series of 29 patients were treated with bevacizumab 5mg/Kg every 14 days, in combination with irinotecan. There were 3 complete responses (CR), 16 partial responses (PR) and 7 stable diseases (SD): thus, 65% of patients achieved a response to treatment. The schedule of most studies that followed was based on a treatment with bevacizumab 10mg/Kg on a once every 14-day schedule, based on the plasma clearance half-life of approximately 21 days. In an early phase II trial, 9 recurrent patients with malignant gliomas and 23 with glioblastomas were treated with a combination of bevacizumab and irinotecan [35]. Twenty patients (63%) achieved a radiographic response to treatment. Of the 23 glioblastomas, 14 patients (61%) achieved a partial response or better, with a median PFS of 20%. In another retrospective analysis of bevacizumab combined with cytotoxic chemotherapy (irinotecan, carboplatin, carboplatin with erlotinib, carmustine or temozolomide) for recurrent malignant gliomas, a total of 55 patients were reviewed and 63% showed a response to treatment and 30% had stable disease [36]. The results of this trial included a 6-month PFS of 42% for glioblastoma and 32% for anaplastic glioma.

In another phase II trial, 48 patients with recurrent glioblastoma were treated with bevacizumab (NCI 06-C-0064E Study) [37]. While on bevacizumab alone, 34 patients (71%) achieved a radiographic response based on Levin's criteria [38] compared to 17 patients (35%) when using MacDonald's criteria [39]. The 6-month PFS was 29%, and 6-month OS was 57%. Nineteen patients were treated with bevacizumab and irinotecan at progression, and no radiographic responses were observed. In a larger multi-institutional phase II trial of bevacizumab alone or in combination with irinotecan for recurrent glioblastoma (Brain Study), 167 patients were randomly assigned to bevacizumab alone or combined with irinotecan [40]. For those patients on bevacizumab alone, 6-month PFS was 43%, objective response rate 28% and median OS was 9 months. For those patients on bevacizumab and irinotecan, 6-month PFS was 50%, objective response rates were 38%, and median OS was 8.7 months.

Overall, when comparing the results of available phase II trials on bevacizumab, alone or in combination with irinotecan, with those of standard cytotoxic chemotherapy in the setting of recurrence (Table1), several findings are clear: bevacizumab alone has activity; bevacizumab clearly increases response rates and PFS at 6 months; bevacizumab does not significantly improve OS so far.

Table 1, Bevacizumab alone or in combination with irinotecan (CPT-11) in recurrent glioblastoma: phase II trial results and comparison with standard treatments

Table **1**, Bevacizumab alone or in combination with irinotecan (CPT-11) in recurrent glioblastoma: phase II trial results and comparison with standard treatments

AUTHOR	DRUG(S)	PFS6 (%)	PFS (WEEKS)	RR (%)	OS (WEEKS)
Friedman et al, 2009	BEV (85)	42	16.8	28	38
Kreisl et al, 2009	BEV (48)	29	16	35	31
Raizer et al, 2010	BEV (50)	25	10.8	30	25.6
Friedman et al, 2009	BEV + CPT-11 (82)	50	22.4	38	36
Yung et al, 2000	Temozolomide (112)	21		8	30
Wong et al, 1999	Miscellaneous (225)	15		6	23
Wick et al, 2010	CCNU (92)	19		4.3	28.4

The improved objective response and PF6 rates observed in the BRAIN and NCI 06-C-0064E studies resulted in May 2009 in the accelerated approval by FDA of single agent bevacizumab for patients with progressive glioblastoma after standard radiotherapy with concomitant/adjuvant temozolomide, whereas EMEA rejected the approval in the European Union due to the lack of an overall survival advantage.

The use of bevacizumab has been shown to decrease both tumoral and peritumoral edema, thereby reducing the requirement for chronic corticosteroid use in 33% to 72% of patients: this effect can be rapid, as early as 18 days after the start of bevacizumab-based therapy [41]. The ability of bevacizumab to taper corticosteroid doses may represent an important clinical benefit, allowing a reduction of side effects, such as Cushingoid pattern of weight gain, hyperglycemia, skin fragility, myopathy, lymphopenia with increased risk of infection, and thromboembolism. Whether this translates in a significant improvement of quality of life remains to be demonstrated properly: the question is extremely important in order to validate a treatment in a palliative setting such as that of recurrent glioblastoma.

Clinical experience suggests that abrupt interruption of anti-VEGF therapy may result in rebound edema and neurological deterioration (the so-called “flare response”): consequently, in stable or responding patients who receive bevacizumab, long-term continuous treatment is often required [42].

Most patients with disease that progressed on a first bevacizumab-containing chemotherapeutic regimen respond poorly to a second bevacizumab and chemotherapy combination [36, 37, 43] with a median PFS of 30-37 days and only few patients had an extended PFS. Conversely, bevacizumab seems to have a role as a salvage therapy following progression in patients treated with VEGF receptor kinase inhibitors [44].

The ideal treatment schedule or dosage of bevacizumab is unclear because no direct comparison of different treatment schedules or dose-response studies have been conducted. A recent phase II single arm study,

employing bevacizumab in an every -3-weeks schedule at 10 mg/Kg, has reported results that are similar to those obtained with the conventional every-2-weeks schedule [45]. A recent meta-analysis of existing literature has been unable to demonstrate a dose-response difference between 5 versus 10 or 15 mg/Kg of bevacizumab with respect to OS, PFS-6 or RR [46].

It is also unclear which chemotherapeutic agent should be combined with bevacizumab to improve efficacy. In fact, none of the drug combinations employed so far have demonstrated superiority over bevacizumab alone. This is true in particular for irinotecan, but also for other drugs investigated less extensively, such as carboplatin, oral etoposide, dose-dense temozolomide, nitrosoureas, cetuximab, erlotinib [36, 47-52]. The combination of bevacizumab with radiotherapy (RT), either in newly diagnosed patients concomitantly with temozolomide [53; 54] or in recurrent tumors employing stereotactic procedures [55] seems safe and feasible. A recent phase II study on 70 patients with newly diagnosed glioblastomas treated with bevacizumab and temozolomide during and after radiotherapy showed improved PFS without improved OS compared to an historical control group of patients who had mostly received bevacizumab at recurrence. Whether bevacizumab added to radiotherapy and temozolomide is able to improve the OS over the standard radiotherapy and temozolomide will be answered by the 2 phase III randomized multicenter trials (RT OG 0825 and Avaglio BO21990), that have just concluded the accrual. More in general, the issue of the optimal timing of bevacizumab use (at diagnosis versus recurrence) remains unclear [57].

Due to it's the ability to reduce vasogenic edema, bevacizumab could be useful in the management of patients with suspected pseudoprogression after combined radiotherapy and temozolomide and in patients with inoperable large tumors to minimize the risk of increasing intracranial pressure when undergoing radiotherapy: a phase II trial in France is investigating the role of neoadjuvant/adjuvant bevacizumab + irinotecan in inoperable glioblastomas.

It is not clear so far whether any subgroup of patients could benefit most from bevacizumab. Interestingly, a retrospective review on 44 patients with recurrent glioblastoma [58] has reported that patients aged 55 years or older and those with a Karnofsky Performance Status of 80 or less had an improved PFS when treated with bevacizumab. A possible explanation was that VEGF expression in glioblastoma specimens was higher among patients older than 55 years of age.

A phase II study in Switzerland is investigating the role of bevacizumab in elderly patients.

Bevacizumab could be of some efficacy in recurrent grade III gliomas [59].

The main mechanism of action of bevacizumab consists in a transient normalization of the tumor vasculature and tumor-induced cerebral edema. The normalization of tumor vessels may reduce tumor hypoxia and enhance the delivery of concurrently administered cytotoxic drugs and radiation. Another potential antitumor mechanism could be targeting of the stem cell-like cancer cells in the vascular niche.

Targeting Angiogenesis through Receptor Tyrosine Kinase Inhibition

In contrast to bevacizumab and aflibercept, which act through ligand sequestration, a large number of agents have been developed that act as competitive inhibitors of VEGF receptors and or other receptor tyrosine kinases for various proangiogenic factors.

The PDGFR inhibitor imatinib failed to show significant survival benefits as monotherapy in several phase I/II clinical trials in patients with recurrent malignant gliomas [60]. However, imatinib in combination with hydroxyurea demonstrated moderate survival benefits in a patient series and two clinical trials of patients with recurrent malignant gliomas [61-63]: in particular a phase II trial reported a PFS6 of 27%. Newer

PDGFR inhibitors, such as tandutinib, might potentially be more effective due to improved blood– brain barrier penetration.

Although the VEGF pathway is critical to angiogenesis, multiple other angiogenic pathways exist, and it is unlikely that inhibition of a single growth factor will result in complete inhibition of angiogenesis. Multikinase inhibitors can potentially inhibit multiple angiogenic growth factor pathways simultaneously by inhibiting several receptors and potentially have greater efficacy. Examples include AZD2171 (cediranib) (pan-VEGFR, PDGFR, and c-Kit inhibitor), pazopanib (VEGFR, PDGFR, and c-Kit inhibitor), sorafenib (Raf, VEGFR, PDGFR, c-Kit, and Flt-3 inhibitor), sunitinib (VEGF, PDGFR, c-Kit, and Flt-3 inhibitor), vatalanib (pan-VEGFR, PDGFR, and c-Kit inhibitor) and XL/184 [64]. Other multikinase inhibitors inhibit angiogenic as well as other tumor cell signaling pathways and may have greater anti-tumor efficacy, such as AEE788 (VEGFR and EGFR inhibitor), dasatinib (Src, VEGFR, PDGFR, Bcr-Abl, and Flt-3 inhibitor), and vandetanib (VEGFR and EGFR inhibitor). Combinations of anti-angiogenic targeted agents may increase effectiveness by inhibiting parallel angiogenic pathways (e.g. vatalanib and imatinib combination targeting the VEGF and PDGF pathways), inhibiting a specific angiogenic pathway at different levels (e.g. bevacizumab and sorafenib combination targeting the VEGF pathway) or by inhibiting an angiogenic and a glioma cell signaling pathway simultaneously (e.g. vandetanib and sirolimus combination targeting the VEGF and PI3K/Akt pathway or sorafenib and temsirolimus combinations targeting the MAPK, VEGF, and PI3K/Akt pathways) [65]. All these agents are being investigated in clinical trials in malignant gliomas.

Encouraging results have come from a recent phase II trial of cediranib in 31 patients with recurrent glioblastoma [66]. Partial responses on MRI were observed in 56,7% and 27% of patients respectively according to the type of measurement (volumetric versus two-dimensional), with a PFS6 of 25.8%, median PFS of 117 days and median OS of 227 days. Corticosteroids were reduced in 66% of patients and discontinued in 33%. A phase III randomized trial in recurrent glioblastomas, comparing cediranib alone vs cediranib + CCNU vs CCNU+ placebo, has been completed but results have not been published yet.

The mechanism action of cediranib is similar to that of bevacizumab. In addition, as observed in preclinical models of glioblastoma treated with cediranib, edema alleviation may result in prolonged survival even without inhibition of tumor growth [67].

The few published data regarding other multitarget agents are up to date disappointing.

Phase I/II trials of vatalanib, in recurrent malignant gliomas have reported low response rates and modest survival benefits as monotherapy or as combination therapy with temozolomide or lomustine (CCNU) [68]. Alone, vatalanib has a 4% partial response (PR) rate and a 12 week median duration of stable disease. In combination with chemotherapy, an 8% PR rate was seen and the time-to-progression (TTP) was 12.1 weeks with lomustine and 16.1 weeks with temozolomide.

A recent phase II study of sunitinib [69] in 21 patients with progressive high-grade gliomas reported an absence of objective responses, a median PFS of 1.6 months and a median OS of 3.8 months. Another phase II trial with pazopanib in 35 patients with a recurrent glioblastoma [70] reported a partial response in 6%, a median PFS of 12 wks, a PFS 6 of 3% and a median OS of 35 wks. The addition of sorafenib to temozolomide in the adjuvant part of the Stupp regimen does not seem to be active [71].

Other Antiangiogenic Approaches

Enzastaurin is an oral inhibitor of protein kinase c-b (PKCb). In a phase II study in heavily pretreated patients with recurrent glioblastoma an interim analysis showed an objective radiographic response rate of

approximately 20%. On the basis of these encouraging data a randomized phase III study was initiated to compare the efficacy of enzastaurin versus lomustine (CCNU) in recurrent glioblastoma, but the trial was stopped prematurely due to the disappointing results on interim analysis. The final study showed no significant difference in any of the efficacy end-points [72]. Response rate, PFS and OS after enzastaurin were median 29%, 1.51 and 6.60 months compared to 4.3%, 1.64 and 7.13 month after CCNU respectively. It is unclear whether enzastaurin is more suitable for combination therapy, especially with radiation therapy.

Cilengitide, an inhibitor of avb3 and avb5 integrins, that has an anti-invasion and anti-migration effect in addition to the anti-angiogenic activity, has shown only modest activity as single agent in recurrent glioblastomas [73]. However, cilengitide may have greater activity combined with radiation therapy and temozolomide in patients with newly diagnosed glioblastomas and MGMT promoter methylation, as suggested by a phase I-II study [74] in 52 patients PFS6 was 69% with an improvement of approximately 15% over radiation with concomitant/ adjuvant temozolomide [1]. The benefits of adding cilengitide to the standard treatment appeared to be limited to patients with glioblastoma with MGMT promoter methylation. The EORTC has launched a phase III trial comparing cilengitide with standard therapy in newly diagnosed glioblastoma patients with MGMT promoter methylation, and now the study has reached the predetermined accrual.

Another antiangiogenic strategy is the so called metronomic chemotherapy. A continuous or near continuous treatment below the maximum tolerated dose of a cytotoxic agent, may limit endothelial cell recovery inhibit the activity of circulating endothelial precursors and up-regulate thrombospondin [75]. In this regard a recent phase II study from Canada has reported that rechallenge with continuous dose-intense temozolomide in recurrent glioblastoma after standard temozolomide + radiotherapy yields an interesting PFS of 23.9%, being patients with either early or late progression those benefiting most [76].

Patterns of Tumor Progression and Resistance to Antiangiogenic Therapies

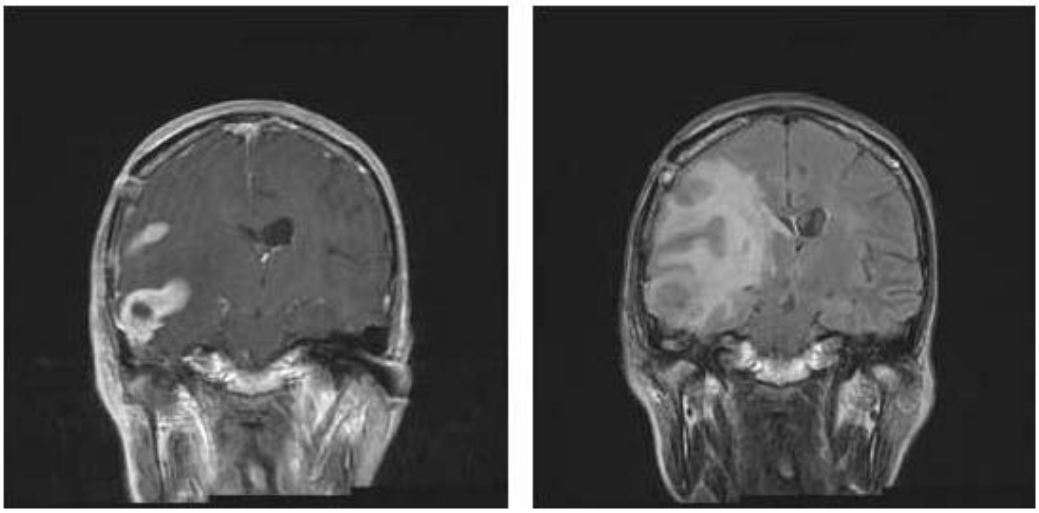
Recurrent glioblastomas invariably relapse after initial response to anti-VEGF therapy. There are two prevailing hypotheses on how these tumor escape anti-angiogenic therapy: vessel cooption and switch to VEGF-independent angiogenic pathways. Preclinical studies have indicated that anti-VEGF therapies might increase the tendency of tumor cells to invades and migrate through a mechanism of cooption of pre-existing vessels [77-79]. This in humans could lead to a nonenhancing pattern of tumor progression on MRI, that is best seen in T2 or FLAIR images, and has been documented in biopsies [80] and autopsies [81] obtained after failure of treatment with bevacizumab. Di Tommaso et al [81] analyzed autoptic tissues from 5 patients recurrent after cediranib versus 7 patients who received no therapy or chemo-radiation but no antiangiogenic agents. They found that after treatment with cediranib endothelial proliferations and glomeruloid vessels were decreased, and vessels diameters and perimeters were reduced to levels comparable to the unaffected controlateral hemisphere. In addition, tumor endothelial cells expressed molecular markers specific to the blood-brain-barrier, indicative of a lack of revascularization despite the discontinuation of therapy. Moreover, the results showed that, instead of switching to alternative angiogenesis pathways, glioblastomas exhibit a more infiltrative phenotype after antiangiogenic therapy. How these data translate into the clinical setting? Some series have reported, following treatment with bevacizumab, an excess of diffuse and/or distant non-enhancing progressions (“gliomatosis-like”), [36, 82-84]. Conversely, two other recent studies, that were more rigorous in measuring the non-enhancing component, did not confirm the preliminary findings. Wick et al [85] have reported that the risk of secondary gliomatosis after bevacizumab (25%) was not significantly higher than with anti-VEGF free regimens (18%). Pope et al [86] evaluated the patterns of recurrence in patients who had received bevacizumab while participating in the BRAIN study. They concluded that the majority of patients did not have a shift in patterns of progression; moreover, patients who had local-to-local or local-to-diffuse progression patterns had similar outcomes, including objective response, PFS and OS. It is possible that treatment with bevacizumab might simply allow the diffuse pattern

to become evident by reducing the enhancement mass and the associated edema and by providing time for the unchecked infiltration to become evident [87].

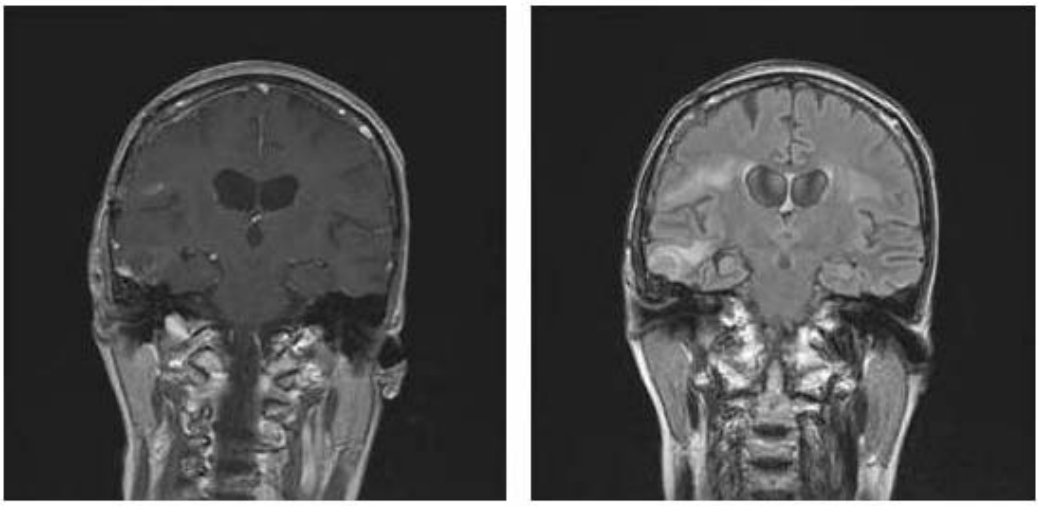
Assessment of Response and Biomarkers

Antiangiogenic agents, especially those targeting VEGF such as bevacizumab or cediranib, can produce marked and early decrease in contrast enhancement, and result in high radiologic response rates of up to 60%. However, these responses may reflect a normalization of vessel permeability and not necessarily be indicative of a true anti-glioma effect. In fact, there is a discrepancy between the high response rates and the modest survival benefit in all series published so far. Moreover, some patients can display a reduction of contrast enhancement on MRI, while progressing in the nonenhancing part of the tumor ("pseudoresponse"). In this regard conventional MacDonald's criteria for the evaluation of response in phase II trials on cytotoxic agents [39] present a major limitation, i.e. they do not take into account the nonenhancing disease in defining the response [88]. This has led to a revision and update of response criteria for high grade gliomas, made by an international panel of experts (Response Assessment in Neuro-Oncology Working Group-RANO). According to the RANO criteria the definition of response (CR or PR), includes, in addition to a measureable disappearance or $\geq$ 50% decrease of the enhancing tumor, at least a stability of the nonenhancing tumor on the T2 / FLAIR images [89] (Fig. 1-2). On the other hand, the sole increase of the nonenhancing part of the tumor can qualify for tumor progression.

To date, there are no validated biomarkers of the response to anti-VEGF therapy, that allow to predict benefit versus lack of benefit early during the treatment course. Among advanced neuroimaging techniques, diffusion MRI seems promising, both to predict response [90] and early progression [91, 92]. PET with fluorothymidime to image proliferation has been suggested of potential usefulness [93]. Multiple molecules in the plasma (VEGF, PIGF, MMP-2 and 10, VEGFR2, sTIE2) and urine (MMP-9), and circulating endothelial cell progenitors are being investigated.



Figures **1a** and **b**, a)Glioblastoma recurrent after standard radiotherapy and temozolomide: baseline T1-weighted image with gadolinium; b)Same as Fig. 1a: FLAIR image.



Figures **2a** and **2b**, a)T1-weighted image with gadolinium after 2 cycles of bevacizumab, showing a partial response of enhancing tumor; b)Same as Fig. 2a: FLAIR image after 2 cycles of bevacizumab, showing a partial response of edema and/or nonenhancing tumor.

TOXICITY OF ANTIANGIOGENIC THERAPIES

Compared to conventional cytotoxic-drugs, antiangiogenic agents are generally well tolerated, but they have unique patterns of toxicity, that require careful selection of patients [94] (Table 2).

Table **2** , Main adverse events after bevacizumab (Grade ≥ 2)

ADVERSE EVENT	**FREQUENCY**
Hypertension	3-15 %
Thromboembolic events (e.g. pulmonary embolism, deep venous thrombosis and stroke)	9-12%

Bleeding complications (e.g. intracranial or pulmonary hemorrhage	0-5 %
Impaired wound healing	0-4 %
Bowel perforation	0-2,5 %
Fatigue	20 %
Headache	10 %
Proteinuria	0-10 %
Transaminitis	5 %
Myelosuppression (e.g. anemia, neutropenia, throbocytopenia)	5-10%
Reversible posterior leukoencephalopathy	5 cases
Optic neuropathy	6 cases

There is an increased risk of thromboembolism in a patient population already at a significant risk of developing deep venous thrombosis, pulmonary embolism, and stroke. Due to the physiological role of VEGF in new blood vessel formation, most anti-VEGF/VEGFR agents are associated with an increased bleeding risk. Although the episodes of bleeding are usually relatively minor, lifethreatening intracranial hemorrhages may occur in a small percentage of patients (≤3%). Because of concerns regarding bleeding, prior intratumoral hemorrhage is generally considered to be a relative contraindication to antiangiogenic therapy.

Despite concerns of hemorrhage with the use of anticoagulants, available data suggests that low molecular weight heparin is relatively safe in patients receiving bevacizumab even if the risk of serous hemorrhages could be higher [95, 96]. Hypertension is a common and dose-limiting toxicity of many anti-VEGF inhibitors, in particular bevacizumab and cediranib, consistent with the physiological role of VEGF in regulating vasomotor tone and blood pressure. Thus, patients need to be carefully monitored, and if necessary, rigorously treated for hypertension.

Other common systemic side effects of VEGF inhibitors are fatigue, proteinuria, epistaxis, impaired wound healing and rarely skin toxicity, and gastrointestinal perforation.

Rare side effects involving the nervous system include reversible posterior leukoencephalopathy.

Optic neuropathy [97] and reversible cognitive deficits after sunitib in patients with preexisting arteriosclerotic leukoencephalopathy [98].

CONCLUSIONS

The role of antiangiogenic therapies in malignant gliomas is emerging. Recent clinical trials and preclinical models have arisen many questions. First of all, it is still to be proven the survival benefit of bevacizumab in both newly diagnosed and recurrent glioblastomas, whereas few data are available regarding anaplastic (grade III) gliomas. The EORTC will launch soon two randomized phase II trials to investigate the role of bevacizumab in glioblastomas and anaplastic gliomas failing standard treatments in comparison with lomustine. Overall, we need to better precise whether the benefit of bevacizumab justify its use, given the high cost.

Another open issue is the best timing for combination of bevacizumab with other anti-neoplastic drugs ("therapeutic window") and timing of hypoxia changes in the tumor after bevacizumab: one cannot exclude that in certain circumstances the vessel normalization could lead to a reduction instead of an improvement of

drug delivery and/or to an increase instead of a decrease of hypoxia, thus increasing instead of reducing chemo-resistance and /or radioresistance.

We urgently need therapeutic strategies after failure of bevacizumab: patients with a rebound of angiogenesis ("enhancing recurrences") and those with a gliomatosis-like nonenhancing pattern of progression probably must be treated with drugs with different mechanisms of action.

Well designed clinical trials and strong translational and basic science studies will probably increase our knowledge regarding the impact of novel targeted therapies on tumors with such a dismal diagnosis.

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CONFLICT OF INTEREST

No conflicts of interest in preparing the manuscript.

ABBREVIATIONS

VEGF = vascular endothelial growth factor
VEGFR = vascular endothelial growth factor RECEPTOR
BM = basement membrane
ECM = extracellular matrix
HIF-1a = hypoxia inducible factor-1a
EGF = epidermal growth factor
EFGR = epidermal growth factor receptor
PTEN = phosphatase and tensin homolog
FGF = fibroblast growth factor
FGFR = fibroblast growth factor receptor
PDGF = platelet derived growth factor
PDGFR = platelet derived growth factor receptor
HGF/SF = hepatocyte growth factor/scatter factor
IL-6 = interleukin-6
IL-8 = interleukin-8
IGF = insulin-like growth factor
IGFR = insulin-like growth factor receptor
TKIs = tyrosine kinase inhibitors
OS = overall survival
PFS = progression free survival

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