Targeted therapy of glioblastomas: a 5-year view

The treatment of glioblastomas requires a multidisciplinary approach that takes the presently incurable nature of the disease into consideration. Treatments are multimodal and include surgery, radiotherapy and chemotherapy. Current recommendations are that patients with glioblastoma should undergo maximum surgical resection followed by concurrent radiation and chemotherapy with the novel alkylating drug temozolomide, followed subsequently by additional adjuvant temozolomide for a period of up to 6 months. We describe here the major signaling pathways that can be constitutively activated in migrating glioma cells, and which render these cells resistant to pro-apoptotic insults such as conventional chemotherapies. In light of this resistance, we therefore describe the molecular therapies and local drug delivery systems that could be used to complement conventional treatments. We have reviewed more than 400 ongoing clinical trials with respect to these new targeted therapy approaches alone or in combination for glioblastoma therapy.

KEYWORDS: angiogenesis inhibitors ▪ clinical trials ▪ glioblastomas ▪ multikinase inhibitors ▪ receptor tyrosine kinase inhibitors ▪ small-molecule inhibitors ▪ targeted therapy

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Patients with glioblastoma (GBM) should undergo maximum surgical resection followed by concurrent radiation and chemotherapy with the alkylating drug temozolomide (TMZ), followed subsequently by additional adjuvant TMZ for a period of up to 6 months [1–3]. In this article we discuss the molecular therapies and local drug delivery systems that could be used to complement conventional treatments.

Epidemiology

Gliomas account for more than 50% of all brain tumors and are by far the most common primary brain tumors in adults [4]. GBMs account for approximately 50% of all glial tumor types and are the type associated with the worst prognosis [2,4]. For reasons that are not clear the incidence of malignant gliomas seems to be rising in elderly people [5]. Locoregional extension, invasion and, less frequently, leptomeningeal dissemination are the main causes of resistance to surgery and adjuvant therapy [4]. At the time of diagnosis, the tumors are occasionally multifocal, even without apparent continuity between the lesions [4].

State-of-the-art at the clinical level

Malignant gliomas, of which GBMs represent the upmost grade of malignancy, continue to remain incurable, and the aim of multimodal treatment is to improve neurological deficits and to increase survival, while maintaining the best possible quality of life [1,2]. The standard treatment for GBM is surgery followed by radiotherapy and chemotherapy. Mounting evidence suggests that a more extensive surgical resection is associated with longer life expectancy for high-grade gliomas [6–8]. The extent of tumor removal and the residual tumor volume correlate significantly with median tumor progression and survival time [7–9]. A tumor removal extent of more than 50% of the total tumor volume is associated with a median time to progression of between 30 and 50 weeks. By contrast, a tumor removal extent inferior to 25% of the total tumor volume is associated with a median time to progression of only 15 weeks [8]. However, quality of life and morbidity are issues.

Fractionated radiotherapy at a total dose of 60 Gy has been shown to prolong the median survival of patients with GBM for an additional 6–8 months, and is the standard adjuvant therapy for high-grade astrocytomas [10,11]. However, up to 90% of all GBMs relapse close to the targeted volume of postoperative radiotherapy [12]. A major step forward in glioma chemotherapy is offered by TMZ, a second-generation imidazotetrazine alkylating agent. TMZ is a small lipophilic molecule, which can be administered orally and which crosses the blood–brain barrier effectively. Moreover, TMZ is less toxic to hematopoietic progenitor cells than conventional

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chemotherapeutic agents and does not require any hepatic metabolism for activation [13]. An international clinical trial conducted by Stupp and colleagues has recently shown that the addition of TMZ to radiotherapy increases the survival of patients suffering from newly diagnosed GBMs [3]. These clinical data strongly suggest that the delivery of TMZ to GBM patients as soon as radiotherapy begins followed by adjuvant TMZ, as compared with radiotherapy alone, significantly impacts their survival. Indeed, the survival beyond 2 years for glioblastoma patients who have undergone conventional treatment is below 10%, while it exceeds 20% in the series of patients treated by Stupp and colleagues [3]. The cytotoxicity of TMZ is thought to be mainly due to the formation of O^6-methylguanine in the DNA because of the depletion in the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT), which mispairs with thymine during the next cycle of DNA replication. Hegi *et al.* [14] and Chinot *et al.* [15] have shown that patients with methylation (inactivation) of the promoter region of the *MGMT* gene have had a better prognosis and a higher likelihood of response to chemotherapy regimens than those without this molecular marker. TMZ first induces the autophagic process in glioma cell lines [16,17], an effect that has to be seen as a cellular defensive mechanism against the chemotherapeutic aggression, but the cytotoxic activity of TMZ is due to the induction of late apoptosis [18]. Katayama *et al.* have recently demonstrated in multiple glioma cell lines that TMZ induces an autophagy-associated ATP surge that protects cells and may contribute to drug resistance [19]. These actions of the compound are not contradictory, because at a molecular level, apoptotic and autophagic response machineries share common pathways that either link or polarize cellular responses [20].

Analysis of the failure of conventional therapies

Malignant gliomas are associated with such a dismal prognosis because glioma cells can actively migrate in the brain, often traveling relatively long distances, making them elusive targets for effective surgical management [2,12]. Following surgical resection and the adjuvant treatment of a glioma, the residual tumor cells peripheral to the excised dense cellular tumor core give rise to a recurrent tumor that, in more than 90% of cases, develops immediately adjacent to the resection margin or within 2 cm of the resection cavity [2,12]. Clinical and experimental data demonstrate that invasive glioma cells show a decrease in their proliferation rates and a relative resistance to apoptosis as compared with the highly cellular tumor core, and this may contribute to their resistance to conventional pro-apoptotic chemotherapy and radiotherapy [12,21,22].

Resistance to apoptosis results from changes at genomic, transcriptional and post-transcriptional levels, ultimately affecting the function of proteins, protein kinases and their transcriptional factor effectors. The PTEN/Akt/PI3K/mTOR/NF-κB and the Raf/Ras/MAPK/ERK signaling cascades play critical roles in the regulation of gene expression and prevention of apoptosis [12] (Figure 1). Components of these pathways are mutated or aberrantly expressed in human cancers, notably GBM. The activity of the PI3K/Akt pathway is often upregulated in brain tumors due to excessive stimulation by growth factor receptors and Ras [23–25] (Figure 1). Moreover, GBMs frequently carry mutations in the *PTEN* tumor suppressor gene, which normally negatively regulates the PI3K/Akt pathway [23,24] (Figure 1). Monoclonal antibodies and low-molecular-weight kinase inhibitors of this pathway are the most common classes of agents in targeted GBM treatment. As we will highlight later, most clinical trials with these agents as monotherapies have failed to demonstrate survival benefit [26], and combinations of agents that can antagonize the activation of this pathway have been reviewed in the excellent recent paper by Gonzalez and de Groot [27]. The level of activation of the PI3K pathway is significantly positively associated with both tumor grade and poor clinical outcome, and negatively associated with apoptosis [28]. Narita *et al.* [29] and Choe *et al.* [30] suggest that the PI3K/Akt pathway is a particularly interesting target in cases of GBM with constitutively activated EGF receptor (EGFR) expression, because EGFR signaling via PI3K/Akt modulates the levels of migration of glioma cells [12]. A number of publications have already reported that an aberrantly activated PI3K/Akt pathway renders tumor cells resistant to cytotoxic insults, including those related to anticancer drugs [31,32]. Shingu *et al.* have shown that the inhibition of this pathway restores or even augments the effectiveness of chemotherapy on glioma cells [32,33]. PI3K inhibitors could also be used to reduce the levels of glioma cell migration, a feature that could restore a certain level of apoptosis to these cells [12,31]. Cell survival through Akt signaling also involves the NF-κB pathway, because Akt signals to various cell-death regulators including IKK, which controls NF-κB activity. NF-κB activity plays a dramatic

role in gliomagenesis. Indeed, the NF-κB signaling pathway is constitutively activated in a large proportion of GBMs [34], and constitutive activation of the NF-κB pathway enables GBM (and other cancer cell types) to resist cytotoxic insults [35,36]. The constitutive activation of Akt and NF-κB contributes significantly to the progression of diffuse gliomas, and the activation of Akt may lead to NF-κB activation in high-grade gliomas. We therefore highlight the use of molecular inhibitors to these pathways in targeted GBM treatment. Since GBM represents one of the most angiogenic cancers, we will also highlight the targeting of angiogenesis in GBM therapy.

Nonreceptor protein kinases, such as Src, Janus kinase/signal transducer and activators of transcription (Jak/STAT), focal adhesion kinase (FAK) and protein kinase (PK)C, also play important roles in glioma biology and may provide attractive targets for future therapeutic strategies [27,37]. We will thus highlight PKCβ as an attractive target for chemotherapeutic intervention in the management of GBM.

Targeted therapy

We obtained information on 487 clinical GBM management trials currently underway (as of 20 January, 2009) using the clinicaltrials.gov website [201], which is a service developed by the National Library of Medicine for the US NIH, as a reference from which to discern the following targeted treatments.

Growth factor receptor inhibitors

As illustrated in Figure 1, the PI3K/Akt pathway is activated following the binding of growth factors to tyrosine kinase receptors (TKRs) bound to the cell surface. Growth factor receptors such as EGFR, IGF-1 receptor (IGF-1R), FGF receptor (FGFR), VEGF receptor (VEGFR) and PDGF receptor (PDGFR), whose abnormal functioning leads to the accelerated clinical progression of malignant gliomas, are known to activate the PI3K/Akt pathway and have already been specifically targeted (Figure 1). We highlight the targeting of VEGFR and PDGFR in the subchapter 'Targeting angiogenesis'. Highly specific

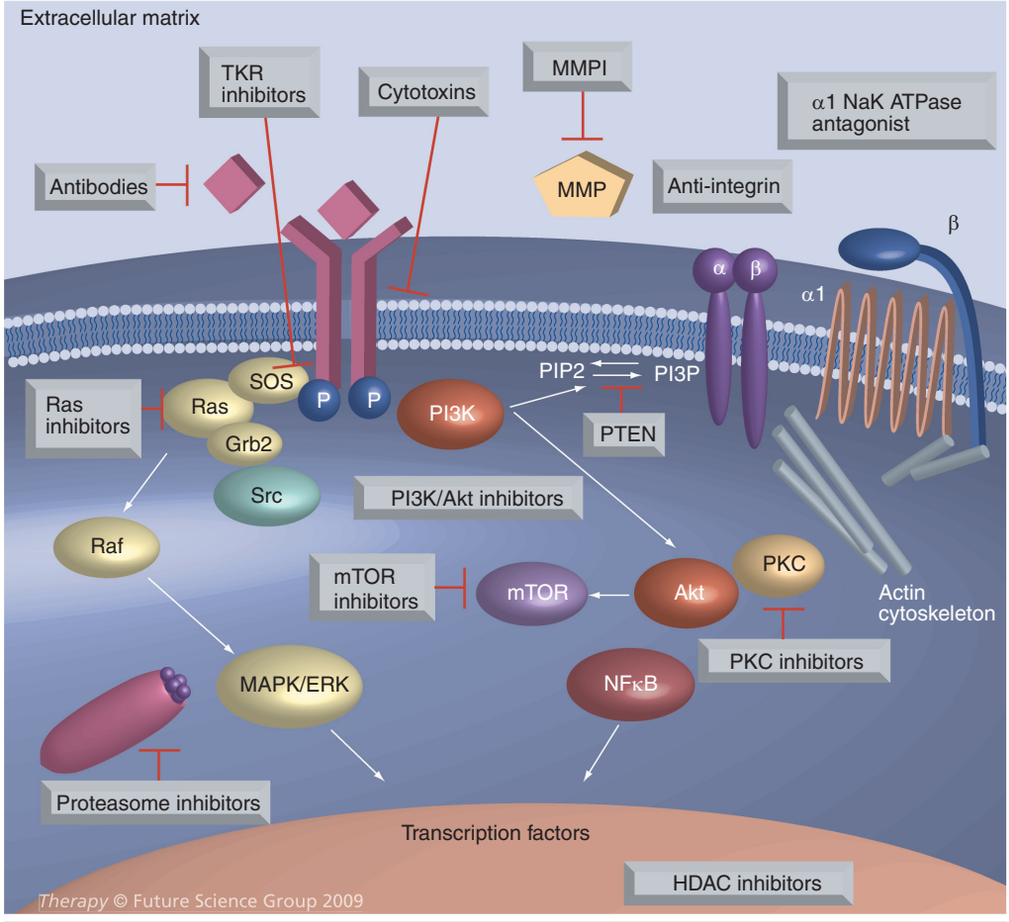

Figure 1. Examples of gliomagenesis pathways serving as the targets for new compounds. HDAC: Histone deacetylase; MMP: Matrix metalloproteinase; PKC: Protein kinase C: TKR: Tyrosine kinase receptor.

small-molecule inhibitors of these TKRs have been developed and may well improve glioma treatment when combined or associated with TMZ [27,38–43] or hydroxyurea [44].

Targeting EGFR

Amplification or overexpression of EGFR is one of the most common molecular abnormalities in GBM [45]. Up to 50% of GBM display amplification of the *EGFR* gene, while a significant proportion of GBM without *EGFR* gene amplification display overexpression of this receptor. Mutant forms of *EGFR* are commonly associated with amplification in GBM. The most common and best characterized *EGFR* mutant (EGFRvIII) results from the deletion of exons 2–7, leading to expression of a truncated receptor [46].

Gefitinib (Iressa®, AstraZeneca, London, UK) and erlotinib (Tarceva®, Roche, Basel, Switzerland) are orally active selective EGFR tyrosine kinase inhibitors that have been undergoing clinical testing with respect to a number of tumors, including malignant gliomas [38–42]. The accelerated approval of gefitinib for non-small-cell lung cancer has been revoked by the US FDA due to the lack of efficacy in published randomized Phase III studies. In the absence of objective responses, some limited antitumor activity was suggested for the treatment of glioblastomas with gefitinib alone [40]. Objective responses were seen in Phase I and II trials with erlotinib alone or given in combination with TMZ for recurrent GBM [41,42]. However, only 10–20% of patients respond to EGFR tyrosine kinase inhibitors. Based on genomic tumor sequencing and immunohistochemistry analysis it seems that the coexpression of *EGFRvIII* and functional PTEN by GBM cells is strongly associated with responsiveness to EGFR kinase inhibitors [47]. Another study suggests that GBM patients who have high levels of EGFR expression and low levels of phosphorylated Akt have better responses to erlotinib treatment than those with low levels of EGFR expression and high levels of phosphorylated Akt [48]. Unfortunately, these results have not been confirmed in larger studies. There was no association between EGFR expression, amplification or *EGFRvIII* mutation and patient outcome when treated by erlotinib as single agent [49,50] or combined with radiation therapy and TMZ [51]. A Phase II study of erlotinib plus TMZ during and after radiation therapy in 65 patients with newly diagnosed GBM reveals a better survival than in those conventionally treated [52]. Median survival was 19.3 months in this study and 14.1 months in the combined historical control studies [52]. The University of California, San Francisco (CA, USA) is running a Phase II study of erlotinib in patients with recurrent EGFR-positive and PTEN functional GBM. Activation of the downstream signaling of Akt and mTOR may be one important factor in the resistance to these agents and justify the combination of several small-molecule inhibitors. Therefore, data suggest that the downstream inhibition of the PI3K pathway, perhaps at the level of mTOR (as detailed below), could be combined with EGFR kinase inhibitors to promote responsiveness in patients with PTEN-deficient tumors [27].

The EGFR-targeting monoclonal antibody nimutuzumab (Oncoscience AG, Wedel, Germany) has demonstrated evidence of no rash (which may make it the only drug inhibiting this pathway that may be useful in a chronic setting) in numerous clinical trials, with a clinical benefit that is equivalent or superior to those of other monoclonal antibodies [53]. Nimutuzumab has entered a Phase III clinical trial for newly diagnosed GBM patients (Table 1). CDX-110 (Celldex Therapeutics, MA, USA) is an immunotherapy that targets the tumor-specific molecule EGFRvIII. Celldex is pursuing the development of CDX-110 for GBM therapy, as well as for other cancers through additional clinical studies. In collaboration with their partner, Pfizer, Celldex is currently performing a Phase II/III randomized, controlled study of CDX-110 combined with standard-of-care versus standard-of-care alone in patients with newly diagnosed GBM (Table 1).

Table 1 summarizes the available clinical trials in the context of recurrent and/or newly diagnosed GBM using EGFR inhibitors.

PI3K/Akt & mTOR inhibitors

The clinical struggle against malignant gliomas should also include inhibitors targeting the signaling pathway controlled by PI3K/Akt (Figure 1, Table 1). Indeed, reducing the signaling abilities of PI3K/Akt would not only reduce the growth levels of malignant gliomas, but should also reduce the migration levels of individual glioma cells invading the brain parenchyma [12,31–33,54]. A reduced migratory capacity in individual glioma cells should render them more sensitive to pro-apoptotic drug treatment (as of current chemotherapies), which they are naturally resistant to whilst migrating [12].

Using the website clinicaltrials.gov, we found only one clinical trial using a PI3K inhibitor, XL765 (Exelixis, CA, USA) combined with

Table 1. Ongoing clinical trials using EGFR receptor inhibitors and PI3K/Akt inhibitors.

Agent	Sponsor	Indication	Stage of development
EGFR receptor inhibitors			
Erlotinib	UNC Lineberger Comprehensive Cancer Center and NCI	Recurrent	Phase I
Erlotinib	Weill Medical College of Cornell University and Genentech	Recurrent/residual	Phase I/II
Erlotinib	The Cleveland Clinic	Recurrent or progressive	Phase II
Erlotinib compared with TMZ or carmustine	EORTC	Recurrent	Phase II
Gefitinib	Duke University and NCI	Recurrent	Phase II
Erlotinib + TMZ and radiotherapy	North Central Cancer Treatment Group and NCI	Newly diagnosed	Phase II
Erlotinib + TMZ and radiotherapy	University of California, San Francisco and Genentech	Newly diagnosed	Phase II
Erlotinib + TMZ and radiotherapy	Case Comprehensive Cancer Center and NCI	Newly diagnosed	Phase II
Cetuximab, radiotherapy and TMZ	University of Heidelberg and Merck KGaA	Newly diagnosed	Phase I/II
Gefitinib + radiotherapy	Radiation Therapy Oncology Group	Newly diagnosed	Phase I/II
CDX-110 (EGFRvIII), radiotherapy and TMZ	Celldex Therapeutics	Newly diagnosed	Phase II
Nimotuzumab	Oncoscience AG	Newly diagnosed	Phase III
PI3K/Akt inhibitors			
XL765 with TMZ	Exelixis	Recurrent	Phase I

EGFR: EGF receptor; EORTC: European Organisation for Research and Treatment of Cancer; TMZ: Temozolomide.

TMZ for recurrent GBM patients (Phase II) (Table 1). XL765 is in fact the first oral dual PI3K and mTOR inhibitor, with Phase I trial results reported by Papadopoulos *et al.* [55]. Hair samples, skin punch biopsies and tumor biopsies obtained before and after drug administration demonstrated decreased phosphorylation of various targets in the PI3K pathway, including Akt.

Several Akt inhibitors are currently in development. Perifosine (Keryx Biopharmaceuticals), an orally bioavailable Akt alkylphospholipid inhibitor, has shown efficacy in preclinical models [56]. However, the clinical Phase II trial for recurrent GBM patients announced by clinicaltrials.gov has been suspended.

An alternate approach has been to use inhibitors of downstream targets within the PI3K/Akt pathway, such as mTOR [54–58]. Bjornsti and Houghton recently reviewed the mTOR pathway as a target for cancer therapy [54]. As emphasized by Sekulie *et al.* [58], the mTOR inhibitor rapamycin is a potent immunosuppressive drug and investigational agent, the major mechanism of action of which involves the inhibition of cell proliferation by blocking cells moving from the G1 to the S phase of the cell cycle. In fact, rapamycin inhibits the phosphorylation of the retinoblastoma protein, and rapamycin-treated cells are therefore not fully committed to entering the S-phase after their release from drug-induced G1 arrest [58]. Constitutive Rb phosphorylation frequently occurs in GBMs. Rapid tumor proliferation (which can result from low apoptotic levels) may contribute to the clinical radioresistance of GBMs, and the disruption of mTOR signaling by rapamycin restores a certain level of radiosensitivity [12]. The modulation of mTOR can also induce autophagy, or type II programmed cell death, a type of cell death to which migrating glioma cells are less resistant as compared with apoptosis [20]. Indeed, mTOR is regulated by mitochondrial dysfunction and the depletion of ATP levels, which can be induced by modifications to cAMP levels or osmotic stress [20], for example.

Inhibitors of mTOR are being extensively evaluated in GBM patients. The main mTOR inhibitors (all rapalogs) currently being assessed are sirolimus (rapamycin, Rapamune®, Wyeth Pharmaceuticals, NJ, USA), temsirolimus

(Torisel®, Wyeth) and everolimus (Certican®, Novartis, Basel, Switzerland) (Table 2). Trials with mTOR inhibitors were first used on patients with recurrent GBMs [59]. However, the results were disappointing [59,60].

The fact remains that all these pathways are not activated at the same time in any single glioma. Particular inhibitors should therefore only be chosen if the target(s) is (are) present in the tumor tissue, and this is only possible if individual patients are submitted to the molecular profiling of their tumors. The stratification of cases based on molecular profiling is currently not exercised in the majority of trials conducted by the National Brain Tumor Consortia funded by the National Cancer Institute, the American Brain Tumor Coalition (NABTC) and the New Approaches to Brain Tumor Therapy (NABTT). The integration of molecular profiling data into clinical practice, such as the 1p19q deletion that identifies glioma patients who will benefit from intensive adjuvant chemotherapy, should be an aim for the future that can be partly accomplished now by compiling all current profiling data.

MAPK & Ras inhibitors

In human GBM, Ras activity is upregulated in the majority of tumors [61]. The ultimate effect of Ras is to induce nuclear transcription via a signaling pathway sequentially involving Raf, MAPK and ERK (Figure 1). To transform cells, Ras oncoproteins must be post-translationally modified with a farnesyl group in a reaction catalyzed by farnesyl protein transferase. Farnesyltransferase inhibitors, therefore, have been proposed as potent anticancer agents targeting Ras. A Phase II study of the farnesyl transferase inhibitor tipifarnib (Zarnestra™, Johnson & Johnson Pharmaceuticals, NJ, USA) in children with recurrent or progressive high-grade glioma, medulloblastoma/primitive neuroectodermal tumor or brain stem glioma, revealed that tipifarnib was tolerated well but had little activity as a single agent [62]. Perillyl alcohol (POH), the isoprenoid of greatest clinical interest, was initially thought to inhibit farnesyl protein transferase. Follow-up studies revealed that POH suppresses the synthesis of small G proteins, including Ras [63]. Intranasal delivery allows drugs that do not cross the blood–brain barrier to enter the CNS, eliminating the need for systemic delivery and thereby reducing unwanted systemic side effects. A Phase I/II clinical trial of POH was performed in patients with relapsed malignant gliomas after the standard treatment of surgery, radiotherapy and chemotherapy. The objective was to evaluate toxicity and progression-free survival after 6 months of treatment. The cohort consisted of 37 patients, including 29 with GBM, five with grade III astrocytoma and three with anaplastic oligodendroglioma. The preliminary results indicate that intranasal administration of the signal transduction inhibitor POH is a safe, noninvasive and low-cost method. There were no toxicity events and the reduction of tumor size in some patients is suggestive of anti-tumor activity [63]. Goldberg and Kloog recently showed that the Ras inhibitor *S-trans*, *trans*-farnesylthiosalicylic acid (FTS) can avert the malignant transformation of human GBM cells by inhibiting both their migration and their anchorage-independent proliferation [64]. They suggest that FTS should be considered as a candidate drug for GBM therapy because it targets not only cell proliferation, but also cell migration and invasion [64]. Table 3 illustrates the ongoing clinical trials using Ras inhibitors including tipifarnib and lonafarnib (Sarasar®, Schering–Plough, NJ, USA).

Targeting angiogenesis

Malignant gliomas represent one type of the most angiogenic cancers. Although several molecular mechanisms contribute to tumor angiogenesis in gliomas, the VEGF pathway appears particularly important and has been a prominent therapeutic target in GBM treatment. This type of approach has recently been reviewed by Puduvalli [65], Lamszus *et al.* [66]

Table 2. Ongoing clinical trials using mTOR inhibitors.

Agent	Sponsor	Indication	Stage of development
Temsirolimus with or without TMZ	Beckman Research Institute NCI	Recurrent	Phase I
Sirolimus	Jonsson Comprehensive Cancer Center and NCI	Recurrent	Phase I/II
Everolimus	Novartis	Recurrent	Phase II
Everolimus and TMZ	NCI of Canada	Newly or recurrent	Phase I
Temsirolimus with TMZ during radiotherapy	North Central Cancer Treatment Group and NCI	Newly diagnosed	Phase I

TMZ: Temozolomide.

Table 3. Ongoing clinical trials using Ras inhibitors.

Agent	Sponsor	Indication	Stage of development
Lonafarnib + TMZ	EORTC	Recurrent	Phase I
Lonafarnib + TMZ	MD Anderson Cancer Center and Schering–Plough	Recurrent	Phase I
Lonafarnib + TMZ	Duke University and shering-Plough	Recurrent	Phase I
Lonafarnib + TMZ	EORTC	Recurrent	Phase I
Tipifarnib + TMZ	MD Anderson Cancer Center	Recurrent	Phase I/II
TLN-4601	Thallion Pharmaceuticals	Recurrent	Phase II
Tipifarnib + TMZ and radiotherapy	North American Brain Tumor Consortium and NCI	Newly diagnosed	Phase I
Tipifarnib + radiotherapy	NCI	Newly diagnosed	Phase II

EORTC: European Organisation for Research and Treatment of Cancer; TMZ: Temozolomide.

and Chamberlain [67]. Several other antiangiogenic agents, such as inhibitors to PDGF, FGF, PKC and integrins are currently in preclinical and clinical development. As emphasized by Sathornsumetee and Rich [68], antiangiogenic therapies remain palliative, suggesting that an effective treatment may require the combination of agents targeting different angiogenic pathways or a multimodality approach that combines antiangiogenic therapy with radiation, chemotherapy, other targeted therapeutics or immunotherapy. Moreover, at present, no predictive biomarkers exist for antiangiogenic therapy.

Targeting VEGF

Strategies for inhibiting the action of VEGF have been developed. VEGF, which increases vascular permeability and stimulates endothelial proliferation and migration, is commonly overexpressed in GBM. As illustrated in Tables 4–7, two strategies have entered clinical practice: ligand-based antagonist therapy utilizing monoclonal antibodies such as bevacizumab (Avastin®, Genentech–Roche) and receptor-based antagonist therapy with tyrosine kinase inhibitors such as AZD2171 (cediranib, Recentin™, AstraZeneca) [69–72]. Early reports suggested an anti-tumor activity for bevacizumab in combination with irinotecan in patients with recurrent malignant glioma [73]. An update on the survival data from this trial was presented at the ASCO annual meeting in May 2008 [74]. The overall response rate for both grades III and IV was 59% (grade III: 61% and IV: 57%). The 6-month period of progression-free and overall survival for grade III were 59 and 79%, respectively, and for grade IV, 43 and 74%, respectively. For grade III and IV patients, the 2-year overall survival rates were 33 and 15%, respectively [74]. Based on these findings, several subsequent studies of this regimen are underway (Table 4). An important feature of bevacizumab is that it shows very good responses as measured by MRI, but there is a debate regarding whether this is really due to reduced tumor size or reduced perfusion. In addition, there is a debate regarding bevacizumab provoking invasion, and this being a resistance mechanism. It seems that bevacizumab plus therapy has become the current treatment of choice for recurrent GBM [67,75]. Three Phase II trials are currently ongoing using bevacizumab with radiotherapy and TMZ in newly diagnosed patients (Table 4).

The only published trial with VEGFR antagonists is using the oral pan-VEGFR tyrosine kinase inhibitor, AZD2171 (cediranib) for recurrent GBM [72]. A Phase II trial is ongoing for newly diagnosed GBM patients using cediranib with radiotherapy and TMZ.

Integrin targeting

Integrins, a family of 24 transmembrane receptors, are named for their ability to integrate extracellular and intracellular activities. They are heterodimers composed of paired α- and β-chains that regulate multiple tumor cell processes, such as angiogenesis, invasion and migration, by mediating cell–cell and cell–extracellular matrix interactions [76]. Integrins are attractive therapeutic targets owing to their increased expression by both GBM cells and tumor vasculature [77]. Initial αvβ3 and αvβ5 integrin inhibitor candidates were primarily antibodies and cyclic or linear peptides [78]. Cilengitide (EMD121974, Merck KGaA, Darmstadt, Germany) is a cyclized pentapeptide (Arg-Gly-Asp-D-Phe-[NMeVal]) designed to block integrin-mediated adhesion and migration. Cilengitide, a selective inhibitor of αvβ3 and αvβ5 integrins with an IC_{50} between 3 and 40 nM, has demonstrated activity in preclinical GBM models. Reardon *et al.* recently reviewed the promising antitumor activities of cilengitide for GBM [79]. The clinical evaluation of cilengitide has proven the

Table 4. Ongoing clinical trials targeting angiogenesis: VEGF targeting.

Agent	Sponsor	Indication	Stage of development
Humanized antibodies blocking the activity of VEGF-A			
Bevacizumab	Robert H Lurie Cancer Center and NCI	Recurrent	Phase II
Bevacizumab + irinotecan	Rigshospitalet Denmark	Recurrent	Phase II
Bevacizumab ± irinotecan	Genentech	Recurrent	Phase II
Bevacizumab + metronomic TMZ	Duke University, Genentech and Schering–Plough	Recurrent	Phase II
Bevacizumab + carmustine	University of California, Davis and NCI	Recurrent	Phase II
Bevacizumab + etoposide	Duke University, Genentech	Recurrent	Phase II
Bevacizumab + TMZ	Duke University, Genentech and Schering–Plough	Unresectable or multifocal	Phase II
Bevacizumab + TMZ following concurrent radio TMZ therapy	University of Chicago and Genentech	Newly diagnosed	Phase II
Bevacizumab + IRI or bevacizumab + TMZ with radiotherapy	Rigshospitalet, Denmark	Newly diagnosed	Phase II
Bevacizumab + TMZ with radiotherapy followed by bevacizumab, TMZ and irinotecan	Duke University, Genentech and Schering–Plough	Newly diagnosed	Phase II
VEGFR tyrosine kinase inhibitors			
Cediranib + lomustine	AstraZeneca	Recurrent	Phase I
Cediranib	Massachusetts General Hospital and NCI	Recurrent	Phase II
CT-322 + irinotecan	Adnexus, a Bristol-Myers Squibb and RD company	Recurrent	Phase II
Cediranib + lomustine	AstraZeneca	Recurrent	Phase III (REGAL)
Cediranib + TMZ and radiotherapy	Massachusetts General Hospital and NCI	Newly diagnosed	Phase I/II
CT-322 + TMZ and radiotherapy	Adnexus, a Bristol-Myers Squibb and RD company	Newly diagnosed	Phase I
Soluble VEGF receptor constructs (VEGF-Trap)			
Aflibercept (VEGF-Trap)	North American Brain Tumor Consortium and NCI	Recurrent	Phase II
Aflibercept + radiotherapy and TMZ	North American Brain Tumor Consortium and NCI	Newly diagnosed or recurrent	Phase I
Small-molecule inhibitors of VEGFR signaling			
Vatalanib (PTK787/ZK222584) + TMZ and radiotherapy	Massachusetts General Hospital, Dana-Farber Cancer Institute and Novartis	Newly diagnosed	Phase I

VEGFR: VEGF receptor; TMZ: Temozolomide.

compound to be initially promising in recurrent GBM patients, and subsequently in newly diagnosed patients. Low toxicity and encouraging activity have been observed among recurrent patients, and synergistic interaction of cilengitide with radiation therapy in preclinical GBM models has also been demonstrated [80] (Table 5). Preliminary results suggest that cilengitide is well tolerated and may improve outcome, particularly for newly diagnosed GBM patients with low MGMT-expressing tumors. Based on encouraging recently reported results [81], a large international randomized Phase III study (Table 5) evaluating the addition of cilengitide to standard TMZ chemoradiation compared with standard TMZ chemoradiation alone for newly diagnosed GBM patients with methylated MGMT tumors started in 2008.

Targeting PDGFR

PDGF and its TKRs (PDGFR) play an important role in angiogenesis. Tumor growth can be promoted by PDGF via autocrine stimulation of malignant cells, by overexpression or

Table 5. Ongoing clinical trials targeting angiogenesis: αvβ3 integrin targeting.

Agent	Sponsor	Indication	Stage of development
Cilengitide	Merck KGaA and EMD Serono	Recurrent	Phase II
Cilengitide, TMZ and radiotherapy	EMD Serono	Newly diagnosed and unmethylated MGMT	Phase II (CORE)
Cilengitide, TMZ and radiotherapy	Merck KGaA and EORTC	Newly diagnosed and methylated MGMT	Phase III (CENTRIC)

EORTC: European Organisation for Research and Treatment of Cancer; MGMT: O^6-methylguanine-DNA methyltransferase; TMZ: Temozolomide.

overactivation of PDGFR or by stimulation of angiogenesis within the tumor. PDGFR blockage may also lower the interstitial fluid pressure within solid tumors and enhance drug delivery. A Phase II study of imatinib mesylate (Gleevec or Glivec, Novartis) in 112 patients with recurrent gliomas of various histologies evaluated the safety and the efficacy of imatinib. The results show that single-agent imatinib is well tolerated but has limited antitumor activity in this patient population [82]. Table 6 illustrates two ongoing trials targeting PDGFR for recurrent GBM patients.

PKC inhibitors

Recent studies have suggested that the proliferation of malignant gliomas may result from activation of PKC-mediated pathways. Activation of PKCβ has now been implicated in tumor cell proliferation, apoptosis and invasiveness. Moreover, activation of PKCβ has been repeatedly implicated in tumor-induced angiogenesis. Enzastaurin (LY317615, Eli Lilly and Company, IN, USA), an acyclic bisindolylmaleimide and an oral inhibitor of PKCβ as well as other isoforms, suppresses angiogenesis and is being advanced for clinical development based upon this antiangiogenic activity [83]. It has been shown that enzastaurin has a direct effect on human tumor cells including GBM cell lines, inducing apoptosis and suppressing the proliferation of cultured tumor cells [84,85]. Enzastaurin treatment also suppresses the phosphorylation of GSK3βser9, ribosomal protein S6 (S240/244) and Akt (Thr308) [84]. Oral dosing with enzastaurin to yield plasma concentrations similar to those achieved in clinical trials significantly suppresses the growth of human GBM and colon carcinoma xenografts [84]. As in cultured tumor cells, enzastaurin treatment suppresses the phosphorylation of GSK3β in these xenograft tumor tissues. Moreover, enzastaurin treatment also suppresses GSK3β phosphorylation to a similar extent in peripheral blood mononuclear cells from these treated mice, suggesting that GSK3β phosphorylation may serve as a reliable pharmacodynamic marker for enzastaurin activity [84]. Along with previously published reports, these data support the notion that enzastaurin suppresses tumor growth through multiple mechanisms: direct suppression of tumor cell proliferation and the induction of tumor cell death coupled to the indirect effect of suppressing tumor-induced angiogenesis [84].

A recent *in vitro* study examined whether the efficacy of enzastaurin could be enhanced through combination with the HSP90 antagonist, 17-AAG, which inhibits Akt and other signaling intermediates by a distinct mechanism [85]. In comparison with the effect of enzastaurin alone, the combination of enzastaurin with 17-AAG led to a marked enhancement of antiproliferative and cytotoxic effects. Simultaneous exposure to both agents significantly increased the release of cytochrome c, as well as caspase 3 activation, Bax cleavage and inhibition of Akt phosphorylation [85]. The authors suggest that the efficacy of enzastaurin can be potentiated by the addition of 17-AAG, and indicate that combining molecularly targeted therapies may provide a more effective strategy than a single-agent therapy to treat patients with malignant gliomas [85].

Table 6. Ongoing clinical trials targeting angiogenesis: PDGFR inhibitors.

Agent	Sponsor	Indication	Stage of development
Imatinib mesylate + TMZ	Duke University and NCI	Recurrent	Phase I
Imatinib mesylate	Novartis	Recurrent expressing PDGFR	Phase II
Tandutinib (FLT3 inhibitor)	NCI	Recurrent	Phase I/II
Dasatinib	Radiation Therapy Oncology Group and NCI	Recurrent	Phase II

PDGFR: PDGF receptor; TMZ: Temozolomide.

Table 7. Ongoing clinical trials targeting angiogenesis: PKC inhibitors.

Agent	Sponsor	Indication	Stage of development
Enzastaurin	National Cancer Institute	Recurrent	Phase I
Enzastaurin and carboplatin	National Cancer Institute	Recurrent	Phase I
Enzastaurin and TMZ	EORTC	Recurrent	Phase I
Enzastaurin	National Cancer Institute	Recurrent	Phase II
Enzastaurin versus lomustine	Eli Lilly and Company	Recurrent	Phase III
Enzastaurin with TMZ during and after radiotherapy	Eli Lilly and Company and University of California, San Francico	Newly diagnosed	Phase I/II
Enzastaurin before, during and after radiotherapy	Eli Lilly and Company	Newly diagnosed	Phase II

EORTC: European Organisation for Research and Treatment of Cancer; TMZ: Temozolomide.

After preliminary Phase I trials established a favorable toxicity profile, enzastaurin has been investigated in completed and ongoing Phase II and III studies in solid and hematologic malignancies, including B-cell lymphomas, where the rationale for its use is the most promising [83]. Indeed, PKCβ was identified by gene-expression profiling, preclinical evaluation and independent immunohistochemical analysis as a rational therapeutic target in B-cell lymphomas, and PKCβ expression was associated with poor outcome and shortened survival in a large independent series of primary B-cell lymphomas [83].

Table 7 illustrates the ongoing clinical trials using enzastaurin alone or in combination with conventional chemotherapy for recurrent and newly diagnosed GBM patients.

Combination of inhibitors & multikinase inhibitors

Response rates using single-agent targeted therapy in GBM have been minimal and the clinical benefit has been difficult to measure. Glioma cells have multiple concomitantly activated tyrosine kinases that lead to activation of multiple signaling pathways [12,75]. Multitargeted kinase inhibitors or combinations of agents targeting different oncogenic pathways may overcome the resistance of tumors to single-agent targeted therapies. Additional clinical studies combine novel targeted therapies with radiation, chemotherapies and immunotherapies. Although the combination of radiotherapy with receptor tyrosine kinase inhibitors was found to be safe in patients with newly diagnosed GBM [86,87], two recent studies did not show a benefit from combining radiation with erlotinib or gefitinib for patients with newly diagnosed GBM compared with historical controls [87,88]. Ongoing studies (Table 8) are therefore evaluating the impact of combining multiple target inhibitors for recurrent GBM and even radiotherapy and TMZ with multiple inhibitors for newly diagnosed GBM patients. Multikinase inhibitors such as lapatinib (Tyverb®/Tykerb®, GlaxoSmithKline, London, UK), which blocks HER2 and EGFR; dasatinib (Sprycel®, Bristol-Myers Squibb, NY, USA), which blocks src, PDGFR, EphA and c-kit; pazopanib (GlaxoSmithKline), which blocks VEGFR1,-2,-3, c-kit and PDGFR; sorafenib (Nexavar®, Bayer, Leverkusen, Germany), which blocks Raf, VEGFR2,-3, PDGFR and Flt-3; sunitinib (Sutent®, Pfizer, NY, USA), which blocks VEGFR, PDGFR, c-Kit and Flt-3; XL184 (Exelixis), which blocks c-met and VEGFR; and vandetanib (Zactima, AstraZeneca) which blocks EGFR and VEGFR, are in clinical trials for recurrent and newly diagnosed malignant gliomas (Table 9). Targeting multiple receptor and nonreceptor kinases using a combination of agents is also being widely pursued. Combining inhibitors of the PI3K pathway with VEGF-blocking agents is attractive, and these studies are already entering into clinical trials. Several studies focus on targeting EGFR and mTOR [89]. Multikinase inhibitors are also combined with other kinase inhibitors and more conventional therapies such as sorafenib combined with an mTOR inhibitor and with radiation and TMZ. A list of clinical trials using combinations of targeted agents and a list of clinical trials using multitargeted agents are shown in Tables 8 & 9, respectively.

Proteasome inhibitors

Critical cellular processes are regulated, in part, by maintaining the appropriate intracellular levels of proteins. Whereas *de novo* protein synthesis is a comparatively slow process, proteins are rapidly degraded at a rate compatible with the control of cell-cycle transitions and cell death induction. A major pathway for protein degradation is initiated by the addition of multiple 76-amino acid ubiquitin monomers via a three-step process of ubiquitin activation and substrate recognition. Polyubiquitination targets proteins for recognition and processing by the 26S proteasome, a cylindrical organelle that recognizes

Table 8. Ongoing clinical trials using a combination of inhibitors.

Agents	Sponsor	Indication	Stage of development
Imatinib mesylate + RAD001 + hydroxyurea	Duke University and Novartis Pharmaceuticals	Recurrent	Phase I
Dasatinib + erlotinib	Duke University, Bristol-Myers Squibb and Genentech	Recurrent	Phase I
Vandetanib with sirolimus	Massachusetts General Hospital, Brigham and Women's Hospital, Dana-Farber Cancer Institute and Astra Zeneca	Recurrent	Phase I
Vorinostat + bevacizumab and irinotecan	H Lee Moffitt Cancer Center and Research Institute and Merck	Recurrent	Phase I
Imatinib mesylate + vatalanib + hydroxyurea	Duke University and NCI	Recurrent	Phase I
Erlotinib + sorafenib, tipifarnib or temsirolimus	North American Brain Tumor Consortium (NCI)	Recurrent	Phase I/II
Erlotinib + temsirolimus	North American Brain Tumor Consortium (NCI)	Recurrent	Phase I/II
AEE788 + everolimus	Jonsson Comprehensive Cancer Center and NCI	Recurrent	Phase I/II
Everolimus + gefitinib	Memorial Sloan-Kettering Cancer Center and NCI	Recurrent	Phase I/II
Sorafenib + temsirolimus	North Central Cancer Treatment Group (NCI)	Recurrent	Phase I/II
Erlotinib + sirolimus	Duke University, Genentech and OSI Pharmaceuticals	Recurrent	Phase II
Enzastaurin + bevacizumab	Eli Lilly and Company and Genentech	Recurrent	Phase II
Enzastaurin + bevacizumab	NCI	Recurrent	Phase II
Erlotinib + sorafenib	NCI	Recurrent	Phase II
Bevacizumab + sorafenib	North Central Cancer Treatment Group (NCI)	Recurrent	Phase II
Cetuximab, bevacizumab and irinotecan	Rigshospitalet, Denmark, Aalborg Hospital and Odense University Hospital	Recurrent	Phase II
Tandutinib + bevacizumab	NCI	Recurrent	Phase II
Bevacizumab + bortezomib	Duke University, Millennium Pharmaceuticals and Genentech	Recurrent	Phase II
Temsirolimus + bevacizumab	Rigshospitalet, Denmark, GCP-Unit, Copenhagen, Wyeth AB, Sweden and Roche, Copenhagen	Recurrent	Phase II
Pazopanib (VEGFR-TKI) + lapatinib (EGFR-TKI)	GlaxoSmithKline	Recurrent	Phase II
Vandetanib + imatinib mesylate + hydroxyurea	Duke University, Novartis and AstraZeneca	Recurrent	Phase II
Vorinostat + bortezomib	North Central Cancer Treatment Group and NCI	Recurrent	Phase II
Tandutinib + bevacizumab	NCI	Recurrent	Phase II
Bevacizumab + erlotinib + TMZ	University of California, San Francisco	Nonprogressive	Phase II
Bevacizumab + erlotinib after radiotherapy and TMZ	Robert H Lurie Cancer Center and NCI	Newly diagnosed	Phase II
Radio, TMZ and bevacizumab followed by bevacizumab/ everolimus	Sarah Cannon Research Institute and SCRI Oncology Research Consortium, and Genentech and Novartis	Newly diagnosed	Phase II

EGFR: EGF receptor; TKI: Tyrosine kinase inhibitor; TMZ: Temozolomide; VEGFR: VEGF receptor.

ubiquitinated proteins, degrades the proteins and recycles ubiquitin (Figure 1). The critical roles played by ubiquitin-mediated protein turnover in cell-cycle regulation makes this process a target for cancer therapy [90]. Bortezomib (Velcade®, Millennium Pharmaceuticals, MA, USA) as the first-in-class proteasome inhibitor has proven to be highly effective in some hematological malignancies, and overcomes conventional chemoresistance, directly induces cell-cycle arrest and apoptosis, and targets the tumor microenvironment [90,91]. It has been granted approval by the US FDA for relapsed multiple myeloma, and recently for relapsed mantle cell lymphoma [90]. Bortezomib sensitizes primary human astrocytoma cells of WHO grades I–IV for TNF-related apoptosis-inducing ligand-induced apoptosis [92].

An *in vitro* study on two human glioblastoma cell lines expressing various levels of EGFR compared gefitinib cytotoxicity with

Table 9. Ongoing clinical trials using multikinase inhibitors.

Agent	Sponsor	Indication	Stage of development
Vandetanib + etoposide	Duke University and Astra Zeneca	Recurrent	Phase I
Sunitinib + irinotecan	Duke University and Pfizer	Recurrent	Phase I
Sorafenib	NCI	Recurrent	Phase I
Vandetanib	NCI	Recurrent	Phase I/II
BIBW2992 (EGFR + HER2/neu) ± TMZ	Boehringer Ingelheim Pharmaceuticals	Recurrent	Phase II
Sorafenib + TMZ	Duke University, Bayer and Schering–Plough	Recurrent	Phase II
XL184	Exelixis	Recurrent	Phase II
Sunitinib	Medical University Innsbruck and Pfizer	Recurrent	Phase II
Sunitinib	Arthur G James Cancer Hospital and Richard J Solove and NCI	Recurrent	Phase II
Sunitinib	NCI	Recurrent	Phase II
Sunitinib	H Lee Moffitt Cancer Center and Research Institute and Pfizer	Recurrent	Phase II
Vandetanib + TMZ during radiotherapy	Dana-Farber Cancer Institute	Newly diagnosed	Phase I/II
Sorafenib adjuvant	Sarah Cannon Research Institute, SCRI Oncology Research Consortium and Bayer	Newly diagnosed	Phase II
Sorafenib concurrent and adjuvant	MD Anderson Cancer Center and Bayer	Newly diagnosed	Phase II

EGFR: EGF receptor; TMZ: Temozolomide.

carboplatin, carmustine and proteasome inhibitor [93]. Among the anticancer agents tested, the proteasome inhibitor bortezomib was the most cytotoxic with a very low IC_{50} on the two cell lines. Bortezomib proved to be a more potent inductor of apoptosis than gefitinib and alkylating agents [93]. However, an *in vivo* study showed that bortezomib, at a clinically relevant dose, did not have any effect on the apoptosis and proliferation of malignant gliomas [94]. These results contrast with the promising preclinical data obtained *in vitro* with this drug [93] and emphasize the importance of performing preclinical studies on animal models, in conditions close to clinical settings.

A Phase I study evaluated the toxicity and response rate of bortezomib with concurrent radiotherapy and TMZ in the treatment of patients with CNS malignancies [95]. A total of 27 patients were enrolled, 23 of whom had high-grade glioma (ten recurrent and 13 newly diagnosed). No dose-limiting toxicities were noted in any dose group, including the highest (1.3 mg/m^2/dose) [95]. All 27 patients were evaluable for response. At a median follow-up of 15.0 months, nine patients were still alive, with a median survival of 17.4 months for all patients and 15.0 months for patients with high-grade glioma [95]. Bortezomib administered at its typical 'systemic' dose (1.3 mg/m^2) was well tolerated and safe combined with TMZ and radiotherapy when used in the treatment of CNS malignancies. Table 10 illustrates the two ongoing phases of clinical trials using bortezomib with tamoxifen or TMZ in the context of recurrent GBM patients.

HDAC inhibitors

Epigenetic modifications are reversible chromatin rearrangements that in normal cells modulate gene expression, without changing DNA sequence. Alterations of this equilibrium, mainly affecting the two interdependent mechanisms of DNA methylation and histone acetylation, are frequently involved in the genesis of cancer [96]. The histone code, which regulates gene expression, is constituted by the combination of different acetylated lysine residues of histones. In neoplastic cells, the abundance of deacetylated histones is usually associated with DNA

Table 10. Ongoing clinical trials using proteasome inhibitors.

Agent	Sponsor	Indication	Stage of development
Bortezomib + TMZ	Beckman Research Institute and NCI	Recurrent	Phase I
Bortezomib + tamoxifen	NCI	Recurrent	Phase II

TMZ: Temozolomide.

Table 11. Ongoing clinical trials using HDAC enzyme inhibitors.

Agent	Sponsor	Indication	Stage of development
Vorinostat (SAHA)	North Central Cancer Treatment Group and NCI	Recurrent	Phase II
Vorinostat + isotretinoin + carboplatin	MD Anderson Cancer Center and Merck	Recurrent	Phase I/II
Vorinostat + TMZ	North American Brain Tumor Consortium and NCI	Non progressive on TMZ	Phase I
Vorinostat + TMZ + radiotherapy	North Central Cancer Treatment Group and NCI	Newly diagnosed	Phase I/II
Valproic acid + TMZ and radiotherapy	NCI	Newly diagnosed	Phase II

SAHA: Suberoylanilide hydroxamic acid; TMZ: Temozolomide.

hypermethylation and gene silencing [96]. Several compounds already known to have *in vitro* antineoplastic activity have been shown to act as histone deacetylase (HDAC) inhibitors. Thus, HDAC inhibitors have been successfully introduced in clinical trials as anti-tumor agents. They are classified according to their chemical structures and the HDACs of classes 1, 2 and 4 are endowed with different specificity and affinity. Among HDAC inhibitors, the most potent are the hydroxamic acid derivatives, such as suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza®, Merck, NJ, USA), which has been recently approved for therapy of cutaneous T-cell lymphomas [96]. SAHA was shown to have potent antiglioma properties *in vitro*, *ex vivo* and *in vivo* [97]. Other classes of HDAC inhibitors are short-chain fatty acids, benzamides, epoxyketone and nonepoxyketone containing cyclic tetrapeptides, and hybrid molecules. short-chain fatty acids, although widely used (especially valproic acid) and clinically efficacious, have weak HDAC inhibition constants [96]. Benzamides, such as MS-275, and cyclic peptides, such as depsipeptide, have been studied in numerous clinical trials and demonstrated low toxicity and significant activity in solid and hematological neoplasms [96]. HDAC inhibitors are also potent radiation sensitizers. In fact, SAHA can enhance radiation-induced *in vitro* cytotoxicity in human prostate and glioma cells [98] and medulloblastoma cells [99]. Moreover, continuous intracranial administration of SAHA inhibits tumor growth in an orthotopic glioma model [100].

The future of HDAC inhibitors in oncology may thus be based on their activity as single agents and on their synergy with the hypomethylating drugs and with chemo- and radiotherapeutics. Table 11 illustrates the ongoing clinical trials using HDAC inhibitors alone or in combination for recurrent as well as newly diagnosed GBM patients.

Targeting IL13 & EGFR receptor

As for EGFR, IL13 receptors α (IL13R-α) are overexpressed in GBM [101]. The presence of IL13 binding sites in GBM and their absence in normal brain tissue validates IL13R-α as an important target in GBM therapy [101,102]. One promising surgical technique for the delivery of drugs directly into the brain parenchyma involves a convection-enhanced delivery system (CED) [103]. CED uses positive pressure infusion to generate a pressure gradient that optimizes the distribution of macromolecules within the tumor and the surrounding tissue. This system is notable in a small number of treatments of recurrent and newly diagnosed high-grade gliomas (Table 12). Rainov *et al.* recently reviewed the clinical trials using CED in the context of GBM therapy [103]. This system has been tried using the drug IL13-PE38QQR (cintredekin besudotox, NeoPharm, IL, USA), a recombinant toxin composed of the enzymatically active portion of *Pseudomonas* exotoxin A conjugated with human IL13 [104]. The binding of the ligand to the receptor (overexpressed or constitutively activated in malignant gliomas) permits the internalization of the recombinant toxin, and this results in a selective and potent cytotoxicity at nanomolar concentrations. Mut *et al.* recently summarized the future of the IL13-targeted cytotoxin [105]. They concluded that the IL13R remains an important potential target in GBM, and preliminary experience with the IL13-PE38QQR cytotoxin has helped to pave the way for study of CED as an important means of drug delivery to GBM [105]. However, the overall survival results from the Phase III PRECISE clinical trial of IL13-PE38QQR cytotoxin delivered via CED in recurrent GBM did not display a statistically significant difference as compared with that of the Gliadel® (wafers containing carmustine) treatment arm [106]. In newly diagnosed

Table 12. Ongoing clinical trials using targeted cytotoxins and metalloprotease inhibitors.

Agent	Sponsor	Indication	Stage of development
Targeted cytotoxins			
IL13-PE38QQR compared with Gliadel Wafer	Neopharm	Recurrent	Phase I (PRECISE trial)
IL13-PE38QQR preoperative	Neopharm	Recurrent	Phase I/II
IL13-PE38QQR after tumor resection + radiotherapy ± TMZ	Neopharm	Newly diagnosed GBM	Phase I
TGF-α *Pseudomonas aeruginosa* exotoxin (TP-38)			
Metalloprotease inhibitors			
Prinomastat + TMZ following radiotherapy	Agouron Pharmaceuticals	Newly diagnosed	Phase II

GBM: Glioblastoma; TMZ: Temozolomide.

GBM, radiotherapy and TMZ seem to enhance the effects of cintredekin besudotox, and this combination is well tolerated [107].

Using the same surgical technique, a recombinant toxin (TP-38) targeting EGFR was also administered to GBM patients [108]. In a study including 20 patients with recurrent GBM, CED-delivered intracerebral TP-38 was well tolerated and produced some durable radiographic responses at doses of less than 100 ng/ml [108]. However, the potential efficacy of drugs delivered by this technique may be severely constrained by ineffective infusion in many patients. Target tissue anatomy and catheter position are critical parameters in optimizing drug delivery [103].

Matrix metalloproteinase inhibitors

Specific antimigratory compounds should be added to conventional radio- and/or chemotherapy. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade some components of the extracellular matrix. A review of MMPs and the development of matrix metalloproteinase inhibitors (MMPIs) can be found [109]. GBM depends on MMPs for tumor cell invasion and angiogenesis. MMPs degrade the basement membrane and the extracellular matrix, thus facilitating tumor growth, invasion, spread and angiogenesis. MMP expression is enhanced in most cancers, including gliomas. Of all the known MMPIs in clinical development, marimastat (British Biotech, Oxford, UK), metastat (CollaGenex, PA, USA), and prinomastat (Pfizer) have been, or are being, tested in trials against gliomas. Combined with TMZ, the MMPI marimastat has yielded the best results to date in Phase II trials, increasing the rate of 6-month progression-free survival in cases of recurrent and progressive GBM that exceeded the literature target by 29% [110]. For all patients, the progression-free survival at 6 months was 39%. Median progression-free survival was 17 weeks, median overall survival was 45 weeks, and 12-month PFS was 16% [110]. More recently, Groves *et al.* showed that even though this regimen is more efficacious than the current standard of treatment as a control in recurrent anaplastic gliomas, the regimen was roughly equivalent to single-agent TMZ and was associated with additional toxicity [111]. Table 12 illustrates the ongoing clinical trial for newly diagnosed GBM patients.

Targeting the sodium pump

Glioma cells are 'self-propelled' [112] and are able to adjust their shape and volume rapidly as they invade the brain parenchyma. Essential to this process is the activity of chloride channels and anion transport mechanisms [113]. The Na^+/K^+–ATPase or sodium pump is another ion transporter that, in addition to exchanging cations, is also directly involved in the migration of cancer cells in general [114,115] and of glioma cells in particular [116]. Accordingly, we have been the first to propose the sodium pump and, more specifically, the α1 subunit of the sodium pump, which is highly expressed in glioma cells compared with normal brain tissues, as a new target in the context of malignant glioma treatment [117].

Using a novel cardenolide with unique structural features [118], which markedly inhibits sodium pump activity and binds to the α1 subunit, we have shown marked antiproliferative and antimigratory effects on human glioblastoma cells (and other cancer cell types) [119]. We have partially unravelled the mechanism of action of this compound, which is to act via the disorganization of the actin cytoskeleton and the induction of autophagic processes in glioblastoma cells [119]. The actin cytoskeleton

is involved in many cellular processes that are essential for cell growth, differentiation, division, membrane organization and motility [12,120]. Moreover, the association of actin filaments with the plasma membrane provides mechanical stability, maintains cell shape and adhesion and regulates dynamic surface protrusions such as lamellipodia and filopodia, which are fundamental determinants of the migratory potential of cells [12,121]. This novel cardenolide recently entered a Phase I clinical trial.

Molecular & genetic profiling of glioblastomas for targeted therapy

As traditional clinical end points prove more difficult to apply in the evaluation of molecularly targeted therapies, a great need exists to define and validate surrogate markers of effect and benefit [122]. Given that the response to TMZ is at least partly associated with low MGMT protein expression [14], MGMT methylation analysis by means of reverse transcriptase-PCR techniques or MGMT immunostaining could be used to predict tumor sensitivity to the drug. However, MGMT methylation is clearly not the only determinant that underlies sensitivity to radiation/TMZ. Recent clinical studies made it clear that targeted therapies may not be effective for all GBM patients, but distinct subsets of patients appear to benefit. As already mentioned before, there was no association between EGFR expression, amplification or *EGFRvIII* mutation and patient outcome when treated by erlotinib as single agent [49,50] or combined with radiation therapy and TMZ [51]. In the same manner, mTOR expression could be evaluated and high tumor mTOR protein levels might indicate suitability for an inhibitor strategy. Most trials using mTOR inhibitors do not measure mTOR levels. Instead the commonly studied biomarkers are usually downstream effectors such as the phosphorylation of ribosomal p70 S6 kinase, which is considered to be a good indicator of the activated Akt/mTOR pathway, as well as rapamycin sensitivity [123]. Similar analyses could also be performed to determine the activation status of other potential biomarkers (PI3-K, Akt and NF-κB) in tumor tissues. Although genome-wide and proteomic profiling of tumors may orient the therapeutic choice, understanding the genotype-response relationships in human tumors will be important for the effective use of targeted therapy in the clinic. The impact of molecular profiling on clinical trial design for GBM has been recently reviewed by Chakravarti and colleagues [124]. New trials will entail a concerted effort to investigate other potential resistance mechanisms in GBM, including key signal transduction, angiogenesis and DNA repair pathways.

Conclusion

The gross-total resection of malignant gliomas is associated with an improved response to adjuvant therapies and consequently, improved survival. New agents as well as advances in delivery systems including CED are likely to have a significant impact on the treatment of malignant gliomas.

It is hoped that together, novel therapies derived from a cellular and molecular understanding of glial tumorigenesis, alongside advances in noninvasive diagnosis, surgical technology and adjuvant treatment, will significantly improve the clinical outcome of these devastating lesions.

Expert commentary

It is imperative that clinical trials that hitherto have focused largely on the intrinsic response of glioma cells to new targeted therapies, shift towards a novel design whereby individual tumor profiling will determine a tailored biomarker-guided treatment that ultimately ensures better efficacy among patients. The need to increase fundamental information on the nature of these cancers in terms of molecular biology is being addressed both through the observations of a European project that will result in the creation of a malignant glioma database and tissue bank, and through ongoing research activities being undertaken by specified groups [125]. However, at present it remains unclear how best to integrate new discoveries in glioma molecular biology into clinical practice [126]. Recent studies have supported the concept that malignant gliomas may be seen as an orchestration of cross-talk between cancer cells, their micro-environment, the vasculature and cancer stem cells. Furthermore, the oncogenic process in such tumors is driven by several signaling pathways that are differentially activated or silenced with both parallel and converging complex interactions. Therefore, it is difficult to identify prevalent targets that act as key promoters of oncogenesis that can be successfully targeted by novel agents [127]. A better strategy may be to identify common molecular abnormalities that are targets of more universally applicable therapies. Thus, novel successes in the fight against certain devastating cancers might be achieved by the combination of pro-autophagic drugs such as TMZ with inhibitors to mTOR, class I PI3-K or Akt, or with endoplasmic reticulum stress inhibitors or antimigratory drugs

as adjuvant chemotherapies [20]. It is probable that improved treatment of these invasive brain tumors will depend on tailoring cocktails of targeted agents to individual patients.

Finally, it is still further hoped that the novel therapies derived from a better cellular and molecular understanding of glial tumorigenesis and of the interaction between these cancers and their microenvironment, alongside advances both in noninvasive diagnosis techniques, including the visualization of tumor tissue by fluorescent methods and in intra-operative monitoring methods that permit more radical tumor resection and adjuvant treatment, will significantly improve the clinical outcome of these devastating lesions.

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Executive summary

- Migrating glioma cells are resistant to pro-apoptotic insults (conventional radiochemotherapies) because of the constitutive activation of the PTEN/Akt/PI3K/mTOR/NF-κB and the Ras/Raf/MAPK/ERK signaling cascades.
- Limited antitumor activity was suggested for the treatment of glioblastomas (GBMs) with EGF receptor (EGFR) tyrosine kinase inhibitors alone.
- Activation of downstream signaling molecules such as Akt and mTOR is one important factor in resistance to the EGFR tyrosine kinase inhibitors and justifies the combination of several small-molecule inhibitors.
- The results of clinical trials with single-agent targeted therapy on patients with GBM were disappointing.
- The VEGF pathway appears particularly important and is a prominent therapeutic target in GBM therapy.
- Antiangiogenic treatments remain palliative, suggesting that overcoming antiangiogenic resistance may require multitargeted kinase inhibitors, a combination of agents targeting different oncogenic pathways or a multimodality combination of pathway inhibitors with radiochemotherapy.
- One promising surgical technique for the delivery of drugs directly into the brain parenchyma involves a convection-enhanced delivery system. This system is used to deliver a toxin either conjugated with human IL13 or targeting EGFR.
- The α1 subunit of the sodium pump (NaK ATPase), which is highly expressed in glioma cells compared with normal brain, could be a new target in the context of GBM therapy.

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