New treatment strategies for malignant gliomas
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Malignant gliomas are the most prevalent type of primary brain tumor in adults. Despite progress in brain tumor therapy, the prognosis of malignant glioma patients remains dismal. The median survival of patients with glioblastoma multiforme, the most common grade of malignant glioma, is 10–12 months. Conventional therapy of surgery, radiation and chemotherapy is largely palliative. Essentially, tumor recurrence is inevitable. Salvage treatments upon recurrence are palliative at best and rarely provide significant survival benefit. Therapies targeting the underlying molecular pathogenesis of brain tumors are urgently required. Common genetic abnormalities in malignant glioma specimens are associated with aberrant activation or suppression of cellular signal transduction pathways and resistance to radiation and chemotherapy. Several low molecular weight signal transduction inhibitors have been examined in preclinical and clinical malignant glioma trials. The efficacy of these agents as monotherapies has been modest; at best; however, small subsets of patients who harbor specific genetic changes in their tumors may display favorable clinical responses to defined small molecule inhibitors. Multitargeted kinase inhibitors or combinations of agents targeting different mitogenic pathways may overcome the resistance of tumors to single-agent targeted therapies. Well designed studies of small molecule kinase inhibitors will include assessment of safety, drug delivery, target inhibition and correlative biomarkers to define mechanisms of response or resistance to these agents. Predictive biomarkers will enrich for patients most likely to respond in future clinical trials. Additional clinical studies will combine novel targeted therapies with radiation, chemotherapies and immunotherapies.

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surgical resection of tumors significantly improves survival [9]. With improvements in neuroimaging and neurosurgical techniques, at initial diagnosis, sequential biopsy followed by resection, in many centers, has been largely supplanted by upfront maximal resection when feasible. Until recently, radiation therapy has been the main standard-of-care treatment following biopsy or resection with minimal utility of systematically administered nitrosoureas. However, Stupp and colleagues reported that adjunctive chemotherapy (concurrent daily temozolomide with radiation, followed by 6 months of monthly temozolomide) increases median survival by 2.5 months without significant degradation in quality-of-life and doubles the number of patients who survive after 2 years of GBM diagnosis [10,11]. Although the survival benefits of adjuvant chemotherapy are modest compared with those observed in other solid cancers, these findings suggest the emerging role of adjunctive chemotherapy and encourage further clinical studies of novel chemotherapy and molecularly targeted therapy for malignant gliomas.

Chemotherapy
Temozolomide (Temodar®, Schering-Plough, NJ, USA) is an orally-available methylating agent that has become the first-line chemotherapy for malignant glioma in the past 5 years; however, the response rate is modest and temporary. In a study of temozolomide for patients with recurrent anaplastic gliomas (WHO grade 3), 8% of patients had a complete response (CR), 27% had a partial response (PR) and 27% had stable disease (SD), with 46% of patients having a PFS of at least 6 months [12]. For patients with recurrent GBM, 5% had a PR and 40% had SD, whereas 6-month PFS was only 21% [13]. Of note, anaplastic oligodendrogliomas, particularly with deletion of chromosomes 1p and 19q, display greater sensitivity to chemotherapies, including temozolomide and the procarbazine/1-(2-chloroethyl)-1-nitrosoureas (CCNU; lomustine)/vincristine (PCV) regimen [14]. Several studies have identified a potential biomarker of clinical response to temozolomide. O6-alkylguanine-DNA alkyltransferase (AGT; also known as O6-methylguanine-DNA methyltransferase [MGMT]) is a DNA-repair protein that removes alkyl groups from the O6 position of guanine, an important site of DNA alkylation. AGT functions as a suicide protein: the reversion of the O6-methylated guanine to an unmethylated state consumes the protein, requiring new protein production. When the temozolomide-induced O6-methylguanine is left unrepaired, cells will undergo futile DNA replication with eventual senescence or apoptosis. High levels of AGT activity in cancer cells create a resistant phenotype by blunting the therapeutic effect of alkylating agents and may be an important determinant of treatment failure [15]. AGT is commonly expressed in low levels in endogenous tissues. Epigenetic silencing of the AGT DNA-repair gene by promoter methylation compromises DNA repair and was associated with longer survival in patients with glioblastoma who received temozolomide [16]. AGT expression may partially inform the selection of patients to receive temozolomide and other alkylating agents. In addition, several Phase I, and ongoing Phase II, clinical trials have demonstrated modest activity of temozolomide in combination with other chemotherapies, such as procarbazine, carmustine (1,3-bis-(2-chloroethyl)-1-nitrosourea [BCNU]), irinotecan (CPT-11), topotecan and etoposide [17-21]. Combinations of temozolomide with molecularly targeted therapies, such as 13-cis-retinoic acid and marimastat (a matrix metalloproteinase [MMP] inhibitor) have also been evaluated [22,23]. The results of these trials, albeit preliminary, showed only modest increases in efficacy when compared with temozolomide monotherapy. Several combination trials of temozolomide with other small molecule inhibitors, such as imatinib mesylate (Gleevec®; Novartis, NJ, USA) [24] or gefitinib (Iressa®; AstraZeneca, DE, USA) are ongoing [25].

Irinotecan (Camptosar®, CPT-11; Pfizer, NY, USA) is a camptothecin derivative that acts as a prodrug, undergoing hydrolysis to the active metabolite SN-38, a potent topoisomerase-I inhibitor [26]. Irinotecan displayed robust antitumor activity against human glioma xenografts and it demonstrated encouraging clinical activity in an early clinical trial [27,28]. However, several Phase II trials of irinotecan from the New Approaches to Brain Tumor Therapy (NABTT), the North Central Cancer Treatment Group (NCTCG) and the North American Brain Tumor Consortium (NABTC) did not show survival benefits in patients with recurrent malignant glioma [29]. A combination of irinotecan and BCNU was associated with additional toxicity without improved therapeutic efficacy compared with irinotecan alone [30,31], whereas the combination of temozolomide and irinotecan in Phase I and II trials displayed acceptable toxicity and encouraging antitumor activity with a 6-month PFS of 29% [19]. Clinical trials with other camptothecin derivatives, such as topotecan and kareakin, are in progress. Other classes of chemotherapy, such as gemcitabine and oxaliplatin, are undergoing additional clinical evaluation. High-dose chemotherapy with stem cell rescue for malignant glioma in adults, unlike for medulloblastoma in children, is feasible but has yet to demonstrate significant efficacy [32,33].

Intra-arterial chemotherapy
Restricted chemotherapy delivery due to the integrity of the blood-brain barrier represents a major challenge in brain cancer treatment. Although central areas of WHO grade 3–4 gliomas frequently demonstrate contrast enhancement on neuroimaging suggesting disruption of normal blood-brain integrity, areas of tumor invading into normal brain remain protected by a vascular barrier. Direct tumor infusion of chemotherapy via a selective cerebral angiography may increase the concentration of chemotherapy in the tumor with limited systemic toxicities. Several strategies have been used to disrupt the blood-brain barrier, such as using osmotic disruption agents (e.g., mannitol) prior to intra-arterial chemotherapy infusion. These approaches may induce significant, transient neurological symptoms, such as cerebral edema. Numerous small Phase I and II studies of platinum-based or vincristine intra-arterial chemotherapy have been completed with evidence of benefit,
although the neuro-oncology community awaits a definitive Phase III study to demonstrate the benefit of intra-arterial chemotherapy over its intravenous counterpart [34].

### Intracavitary/intratumoral

Limited drug delivery and high toxicities associated with systemic chemotherapy have led to different approaches of therapeutic agent delivery. In addition, failure to eradicate local tumor growth is a major factor contributing to poor outcome, as indicated by the development of 80–90% of GBM recurrences within 2 cm of the original resection site [35]. Therefore, direct intratumoral or intracavitary administration of chemotherapy, conjugated biological toxins or radiolabeled monoclonal antibodies may improve local control of tumors in patients with malignant gliomas.

BCNU-impregnated wafers (Gliadel®; MGI Pharma, M N, USA) are the first US FDA-approved biodegradable wafers (polifeprosan 20) containing chemotherapy used in the treatment of malignant glioma. A recent large Phase III trial of 240 patients with newly diagnosed malignant glioma randomized to receive either BCNU- or placebo-impregnated wafers at the time of primary surgical resection, followed by radiation therapy, demonstrated a modest survival benefit with a median survival of 13.9 months for the BCNU wafer-treated group and 11.6 months for the placebo-treated group [36]. Side effects were comparable between the two groups, except cerebrospinal fluid (CSF) leaks and intracranial hypertension, which were more common in the BCNU wafer-treated group. As BCNU wafers followed by radiation therapy treatment may promote necrosis as a treatment effect, BCNU wafer-treated patients with radiographic findings suspicious for local recurrence may demand biopsy for diagnostic clarification [37]. Implantation of BCNU wafers in patients undergoing resection for recurrent GBM only provides a modest 8-week prolongation of survival [38]. Combinations of BCNU wafers with other agents are ongoing.

A radiolabeled monoclonal antibody against tenasin (121I-m81C6) has been developed at Duke University, N C, USA. Tenasin is expressed ubiquitously in high-grade gliomas but not in normal brain. Preclinical studies of this radiolabeled antibody demonstrated significant tumor growth delay and regression in athymic mice bearing subcutaneous human glioma xenografts and prolongation of median survival for athymic rats bearing intracranial tumors [39,40]. In a Phase II trial, 33 patients with newly diagnosed malignant glioma (GBM, n = 27; anaplastic astrocytoma [AA], n = 4; anaplastic oligodendroglioma [AO], n = 2) underwent surgical resection with intracavitary 121I-m81C6 administration, followed by conventional external-beam radiotherapy and 1 year of alkylator-based chemotherapy. Median survival for patients with GBM was 79.4 weeks. A total of 27% of patients developed reversible hematological toxicity and 15% developed histologically confirmed, treatment-related neurological toxicity [41]. A randomized Phase II trial of 121I-m81C6 administration prior to radiation therapy with concurrent temozolomide is ongoing. A Phase III multicenter trial with patient-specific dosing is planned. In addition, 121I-m81C6 was also tested in a Phase II trial of patients with recurrent malignant gliomas. With a median follow-up of 172 weeks, 63 and 59% of patients with GBM and AA/AO tumors, respectively, were alive at 1 year. Median overall survival for patients with GBM and AA/AO tumors was 64 and 99 weeks, respectively [42]. Other localized radiation treatments – radiosurgery (including γ-knife and linear accelerator), 125I beads and intracavitary balloons filled with radioisotope (GliaSite Radiation Therapy System, Cytyc, GA, USA) – have also been used for localized recurrences of malignant gliomas [43,44]. Intracavitary ‘inside-out’ radiation has a theoretical advantage of maximal radiation delivery to the tumor bed with minimized radiation to the normal surrounding brain.

### Cytokine-conjugated toxin therapy

Malignant gliomas commonly overexpress several cell surface receptors that bind ligands and internalize to permit the specific delivery of radioisotopes or toxins into tumor cells. Interleukin (IL)-13 receptors are abundantly expressed on glioblastoma cells but not on normal brain or endothelial cells. Therefore, IL-13-conjugated exotoxin presents a method to specifically kill the tumor cells [45]. A recombinant protein of IL-13 and a mutated form of Pseudomonas exotoxin (IL-13–PE38QQR; cintredekin besudotox, NeoPharm, IL, USA) has been developed. Intracerebral administration of IL-13 cytotoxin to rodents bearing glioma xenografts improved animal survival but failed to demonstrate survival benefit with systemic administration. Based on these encouraging preclinical studies, several Phase I/II clinical trials in adults with malignant glioma have been completed and IL-13 cytotoxin therapy appears to be safe [46]. A randomized Phase III trial of IL-13 cytotoxin versus BCNU wafer has completed enrollment. A Phase I trial of IL-13 cytotoxin in newly diagnosed GBM is ongoing. Other cytokine-conjugated toxin therapies include IL-4-conjugated Pseudomonas toxin that also binds IL-13 receptors [47] and transforming growth factor (TGF)-α conjugated with mutated Pseudomonas toxin (T-P38) that binds epidermal growth factor receptors (EGFRs) [48].

### Targeting chemotherapy resistance

O6-benzylguanine

As aforementioned, gliomas frequently express the DNA repair enzyme AGT (or MGMT), which removes chloroethylation or methylation damage from the O6-position of guanine to promote resistance to DNA toxicity by alkylating agents, such as temozolomide, and the nitrosoureas, such as carmustine and CCNU. Therefore, targeting AGT may overcome chemotherapy resistance in malignant gliomas. O6-benzylguanine (O6-BG) is an AGT substrate that inhibits AGT by suicide inactivation. Preclinical studies suggest the enhancement of chemotherapeutic efficacy by O6-BG in a murine glioma model [49,50]. Several Phase I malignant glioma trials of intravenous O6-BG alone or in combination with carmustine or temozolomide revealed that
AGT was depleted following intravenous administration of O\(^6\)-BG [51,52]. However, a Phase II trial of carmustine plus O\(^6\)-BG failed to demonstrate a clinical response, with more than 50% of patients developed grade 3 or higher hematological toxicities [53]. A Phase II trial of O\(^6\)-BG in combination with temozolomide has been completed.

**Molecularly targeted therapy**

As aberrant signal transduction pathways regulating malignant phenotypes of cancer have undergone elucidation, several novel therapies specifically targeting these molecular abnormalities in glioma cells have been developed. As with other cancers, malignant gliomas share the common essential characteristics - proliferation, avoidance of apoptosis, evasion from immune surveillance, new blood vessel formation and an ability to invade normal tissues [54]. Most GBMs are diagnosed without an antecedent lower-grade tumor being detected - so-called de novo or primary GBMs. A smaller fraction of high-grade astrocytomas, termed secondary GBMs, present initially at a lower grade, with subsequent progression to higher grades. The two presentations of GBM s may be associated with different frequencies of molecular changes (figure 1). Low-grade astrocytomas (WHO grade 2) commonly display mutations of the tumor-suppressor gene TP53 and overexpression of platelet-derived growth factor (PDGF) ligands and their cognate receptors in glioma precursor cells [55]. Progression to AA involves several cellular alterations, including deletion or mutation of p16\(^{INK4A}\) or retinoblastoma susceptibility locus 1 (pRB1), or amplification or overexpression of cyclin-dependent kinase (CDK)4 and human double minute 2 (HDM2; human xenolog of mouse double minute 2 [MDM2]). Progression to grade 4 (i.e., GBM) is associated with deletion of chromosome ten, whereas primary (de novo) GBMs often display loss of phosphate and tensin homolog deleted on chromosome ten (PTEN) and amplification, mutation or overexpression of the EGFR. Other molecular abnormalities in primary GBM s involve mutation or deletion of the tumor-suppressor genes p14\(^{ARF}\) and p16\(^{INK4A}\) that are co-localized on chromosome 9p. In addition, anaplastic astrocytomas [WHO Grade 2] or anaplastic astrocytomas [WHO Grade 3] or, more commonly, present as primary, de novo glioblastomas. Genetic analyses of gliomas have demonstrated differences in common molecular alterations between primary and secondary glioblastomas. Each molecular target may have an important role in cellular transformation and tumor maintenance. No single genetic change defines glioblastoma development, indicating that attacking a single target might not be sufficient to control the growth of the majority of gliomas.

CDK4: Cyclin-dependent kinase 4; EGFR: Epidermal growth factor receptor; HDM2: Human double minute 2; PDGFR: Platelet-derived growth factor receptor; PTEN: Phosphatase and tensin homolog deleted on chromosome ten; RB1: Retinoblastoma susceptibility locus 1.

**Growth factor inhibition**

EGFR, a prototypic receptor of the ErbB family, is amplified in approximately 50% of GBM s and is overexpressed in many malignant gliomas, regardless of amplification status [57]. EGFR gene amplification is also associated with poor prognosis in patients with GBM s [58]. In addition, frequent overexpression of several EGFR mutants, including EGFR\(^{VII}\) (EGFR-\(Δ2–7\), which has a deletion of exons 2–7 causing a deletion in the extracellular ligand-binding domain and constitutive activation in a ligand-independent manner, suggests that EGFR is a key factor in tumorigenesis and provides a compelling rationale for the use of EGFR-targeted therapies in these patients [59]. Two small molecule inhibitors of EGFR, erlotinib (Tarceva\(^®\), OSI-774; Genentech, Inc., CA, USA) and gefitinib (Iressa\(^®\), ZD1839; AstraZeneca, DE, USA) have been tested widely in human malignancies, including malignant gliomas. In the authors’ recently published Phase II trial of gefitinib for recurrent GBM patients, the median event-free survival (EFS) was only 8.1 weeks and no radiographic responses were observed, although nine out of 53 patients (17%) remained progression-free for at least 6 months [60]. No activating mutations of the EGFR kinase region were identified in patients who had no disease progression at 6 months. Similarly, in a Phase I study of erlotinib, radiographic responses were reported in eight out of 41 patients (20%) treated with [61].

**Figure 1. Molecular alterations in malignant gliomas.** Glioblastomas develop from the malignant progression of low-grade tumors (diffuse astrocytomas [WHO Grade 2] or anaplastic astrocytomas [WHO Grade 3]) or, more commonly, present as primary, de novo glioblastomas. Genetic analyses of gliomas have demonstrated differences in common molecular alterations between primary and secondary glioblastomas. Each molecular target may have an important role in cellular transformation and tumor maintenance. No single genetic change defines glioblastoma development, indicating that attacking a single target might not be sufficient to control the growth of the majority of gliomas.

Gliomas are strikingly heterogeneous tumors in terms of their pathology and gene expression, even within a single tumor. Despite the variability, common alterations in specific cellular signal transduction pathways or cellular functions occur within the majority of gliomas, leading to the emerging trials of novel small molecule inhibitors in the clinic (figure 2).
New treatment strategies for malignant gliomas

In a Phase II study of patients with recurrent GBM, four (8.4%) achieved a radiographic response, 18 (37%) achieved SD and the 6-month PFS was 17%. Recent excitement has been generated by an elegant study demonstrating that tumor expression of EGFRvIII and wild-type PTEN was associated with an increased likelihood of radiographic response, although the durability of responses noted was generally limited. These findings may serve as a rationale to stratify patients in future clinical trials involving EGFR-targeted therapy.

PDGF receptors (PDGFRs) are important in growth signaling pathways and neoangiogenesis of gliomas. PDGF autocrine growth signaling is characterized by coexpression of PDGF and its receptors. The PDGF family consists of four members, PDGF-A,
-B, -C and -D, which signal through the α and β PDGFR tyrosine kinases. PDGF ligands and PDGFR-α are expressed in most human gliomas, while PDGFR-β is expressed in glioma cells and tumor endothelial cells [64]. By promoting pericyte recruitment, PDGF-B enhances angiogenesis through an increased expression of VEGF in the tumor endothelial cells [65]. Imatinib mesylate, a small molecule adenosine triphosphate (ATP)-mimetic kinase inhibitor of PDGFR, c-Kit and Bcr-Abl, exhibited antiglioma activity in preclinical studies [66]. In addition, imatinib mesylate also sensitizes glioma cells to radiation injury [67]. Despite the successful preclinical studies, imatinib mesylate monotherapy failed to demonstrate clinical benefits in several Phase II trials [68,69]. By contrast, the combination of imatinib mesylate and hydroxyurea has shown promising results in one patient series [70], which was confirmed by a subsequent Phase II trial in recurrent GBM patients [71]. A total of 33 patients enrolled with progressive disease after radiation therapy and chemotherapy including temozolomide. The 6-month PFS was 27% and median PFS was 14 weeks. 9% of patients had radiographic response, while 42% achieved SD at the median follow-up of 58 weeks [71]. The mechanism of anti-tumor activity of this combination remains unknown. Imatinib mesylate may also act as an inhibitor of multidrug ATP-binding cassette (ABC) transporter type G2 (ABCG2; also known as breast cancer resistance protein [BCRP]) [72]. ABCG2 is a half ATP-binding cassette transporter mediating efflux of several drugs from the tumor cells conferring chemotherapy resistance. In addition, ABCG2 is also expressed abundantly in the blood–brain barrier, inhibiting penetration of drugs into the brain [73]. By inhibiting ABCG2, blood–brain barrier penetration and tumor cell uptake of hydroxyurea may be augmented, leading to increased tumor cytotoxicity. Further studies of this combination in both clinical and preclinical studies are critically required. Given the encouraging results of imatinib mesylate plus hydroxyurea, several combinations of imatinib mesylate with other chemotherapies, such as temozolomide, are under investigation in clinical trials [24].

Vascular endothelial growth factor inhibition

One of the hallmarks of cancer is the ability to form new blood vessels (neangiogenesis) [74]. Vascular endothelial growth factor (VEGF) is a heparin-binding growth factor specific for vascular endothelial cells that plays a pivotal role in angiogenesis [75]. Glioma cells secrete VEGF, whereas tumor-associated endothelial cells express high levels of the cognate receptors, VEGF receptor (VEGFR)2, creating a paracrine loop of angiogenesis activation. Since gliomas are among the most angiogenic cancers, VEGF has been a focus in the development of glioma-targeted therapies. A murine monoclonal antibody to VEGF (A4.6.1) was developed in the early 1990s [76] and, later, its recombinant human counterpart (bevacizumab; Avastin®; Genentech, CA, USA) became the front-line treatment for several advanced solid malignancies [77,78]. In a rat intracranial C6 glioblastoma model, A4.6.1 treatment decreased tumor vascularity, enhanced tumor apoptosis and prolonged survival [79]. The antitumor mechanism of bevacizumab is unclear. Changes in vascular functions were frequently reported, including decreased vessel diameter, density and permeability in response to treatment. A reduction in interstitial fluid pressure has also been observed. In some studies, these improvements resulted in an increase in intratumoral uptake of chemotherapy, implying that the most effective use of anti-VEGF therapy may be in combination with chemotherapy [80]. Although antibodies have been successfully used in localized therapies, the use of such large molecules in glioma therapy has been slowed by the delivery concerns. Targeting the VEGF ligand may be efficacious as only intraluminal vascular delivery is required. Stark-Vance recently reported dramatic efficacy of bevacizumab plus irinotecan for patients with recurrent GBM [81]. In this unpublished case-series of 29 heavily pretreated patients with recurrent GBM, 16 patients achieved radiographic response (55%), ten achieved SD (34%) and only three developed progressive disease (PD; 10%). Overall, the regimen was well tolerated, although one cerebral hemorrhage was reported. A Phase II trial of this combination at Duke has completed enrollment. The preliminary data confirms the robust efficacy of this combination, as observed by Stark-Vance (VREDENBURGH, SUBMITTED). Future studies of bevacizumab in combination with radiation therapy, other chemotherapies or small molecule kinase inhibitors are being developed. Another promising VEGF-targeted agent is a soluble decoy receptor of VEGF (VEGF-trap) [82]. The NABTC will conduct a clinical trial of VEGF-trap in recurrent malignant glioma.

Vascular endothelial growth factor receptor inhibitors

Pharmacological inhibition of VEGFR2, either by a monoclonal antibody or by small molecule inhibitors, has shown efficacy in preclinical models [83–87]. In addition, an antibody to VFGFR2 also restored the sensitivity of a radioresistant glioma cell line to radiation treatment [88]. Based on these encouraging results, several clinical trials of angiogenic kinase inhibitors have been initiated. SU5416 (Pfizer, NY, USA) is a kinase inhibitor of VEGFR and PDGFR that was evaluated in multicentered Phase I/II trials as monotherapy or in combination with chemotherapy. Conrad reported that, for a PTK787/ZK222584 study for recurrent malignant gliomas, out of 47 evaluable patients, there were two PR patients, 31 SD patients and 14 PD patients [89]. Reardon reported a Phase I combination study of PTK787/ZK222584 with either temozolomide or CCNU. The combinations were well tolerated, although the efficacy was limited with a time to progression of 16.1 weeks for temozolomide plus PTK787 and 12.1 weeks for CCNU plus PTK787 [90].

Multitargeted kinase inhibitors

Mechanisms for the initial failure of targeted therapy clinical trials in many solid neoplasms include the pre-existence or development of multiple parallel or compensatory oncogenic pathways permitting tumor survival. Newer generation small molecule kinase inhibitors are designed to target multiple aberrant signaling pathways in tumors and tumor-associated vasculature (BOXES 1 & 2). The disadvantage of multitargeted kinase inhibitors is a general
increase in toxicity, although the efficacy in preclinical studies of these agents appears to be superior to single-targeted kinase inhibitors. Several small molecule kinase inhibitors disrupting EGFR on the tumor and VEGFR2 on the tumor-associated endothelial cells have been developed. AEE788 (Novartis, NJ, USA) is a dual kinase inhibitor of EGFR and VEGFR2 (KDR). AEE788 has efficacy in primary glioma cell lines and a murine model of glioblastoma [91, 92]. Multicentered trials of AEE788 alone or in combination with RAD001 (rapamycin analog; Novartis, NJ, USA) in recurrent malignant glioma are ongoing [93]. ZD6474 (Zactima®; AstraZeneca, DE, USA), a kinase inhibitor of VEGFR2 and EGFR, displayed survival benefits in a murine model of intracranial human glioma xenografts [94] and a clinical trial of ZD6474 in malignant glioma is under development. Sunitinib malate (SU11248; Pfizer, NY, USA), a multitargeted kinase inhibitor of VEGFR, PDGFR, c-kit and fetal liver tyrosine kinase (FLT)-3, has demonstrated activity in a subcutaneous model of glioblastoma [95]. A Phase I trial of SU11248 in advanced solid malignancies has been completed and a Phase II study in malignant glioma is planned [96]. Sorafenib ( Nexavar®, BAY 43–9006; Bayer, CT & O nyx, CA, USA) is a kinase inhibitor of RAF, VEGFR and PDGFR that exerts broad-spectrum antitumor activity in several cancer cell lines [97]. Although most GBM s lack RAF mutations, targeting RAF/M/EK/ERK pathway may be beneficial by its universal antiproliferative effects. Sorafenib is well tolerated in a Phase I study in advanced solid tumors [98]. The NABTC is conducting multiple Phase I/II studies of sorafenib in combination with erlotinib, the farnesyltransferase inhibitor, tipifarnib (R115777; Zarnestra®; Johnson & Johnson, NJ, USA) or the mammalian target of rapamycin (mTOR) inhibitor, temsirolimus (CCI-779; Wyeth, PA, USA). Lapani (GW572016; EGFR and HER2 inhibitor) ZD6474 (Zactima®: EGFR and VEGFR inhibitor) Lapatinib (GW572016; EGFR and HER2 inhibitor) Sorafenib (Nexavar®, BAY 43–9006; RAF, VEGFR and PDGFR inhibitor) Farnesyltransferase inhibitors Tipifarnib (Zarnestra®; R115777) Lonafarnib (Sarasar®; SCH66336) Akt inhibitor Perifosine (KRX-0401) mTOR inhibitors AP23573 Sirolimus (rapamycin; rapamune®) Temsirolimus (CCI-779) Everolimus (RAD001) HDAC inhibitors Suberoylanilide hydroxamic acid (SAHA) Depsipeptide (FK228) Hsp-90 inhibitor 17-Allylamino-geldanamycin (17-AAG) PKC inhibitors Tamoxifen Enzastaurin (LY317615) Agents targeting invasion Cilengitide (EMD121974) (αvβ3 and αvβ5 integrin inhibitor) Marimastat (MMP inhibitor) Apoptosis-modulating agents Recombinant human TRAIL All-trans retinoic acid EGFR: Epidermal growth factor receptor; HDAC: Histone deacetylase; Hsp: Heat-shock protein; IL: Interleukin; mTOR: Mammalian target of rapamycin; MMP: Matrix metalloproteinase; PDGFR: Platelet-derived growth factor receptor; PI3K: Phosphatidylinositol 3-kinase; PKC: Protein kinase C; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; VEGFR: Vascular endothelial growth factor.

**Box 1. Summary of targeted therapeutic agents in clinical trials of malignant gliomas.**

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<tr>
<th>EGFR inhibitors</th>
<th>Gefitinib (Iressa®; ZD1839)</th>
<th>Erlotinib (Tarceva®; OSI-774)</th>
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<tr>
<td>PDGFR inhibitor</td>
<td>Imatinib mesylate (Gleevec®; STI571)</td>
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<td>VEGFR inhibitor</td>
<td>Bevacizumab (Avastin®; recombinant human VEGF antibody)</td>
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<tr>
<td>Multitargeted kinase inhibitors</td>
<td>Vatalanib (PTK787/ZK222584; PDGFR and VEGFR inhibitor)</td>
<td>Sunitinib malate (SU11248; PDGFR and VEGFR inhibitor)</td>
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<tr>
<td>Farnesyltransferase inhibitors</td>
<td>Tipifarnib (Zarnestra®; R115777)</td>
<td>Lonafarnib (Sarasar®; SCH66336)</td>
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<td>Akt inhibitor</td>
<td>Perifosine (KRX-0401)</td>
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<td>PKC inhibitors</td>
<td>Tamoxifen</td>
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<tr>
<td>Agents targeting invasion</td>
<td>Cilengitide (EMD121974) (αvβ3 and αvβ5 integrin inhibitor)</td>
<td>Marimastat (MMP inhibitor)</td>
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<td>Apoptosis-modulating agents</td>
<td>Recombinant human TRAIL</td>
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<td>All-trans retinoic acid</td>
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been found in glioma cells compared with normal brain and/or normal astrocytes [103,104]. Major roles in tumor astrocyte migration are played by α3β1, α5β1, αvβ3 and αvβ5 integrins. Cilengitide (EMD 121974; EMD pharmaceuticals, NC, USA), an intravenous inhibitor of αvβ3 and αvβ5 integrins, demonstrated preclinical activity in malignant gliomas [105]. Cilengitide induces apoptosis in experimental U87MG gliomas by preventing them from adhering to vitronectin and tenascin, which are matrix proteins known to be essential for brain tumor invasion and growth [106]. The NABTT group conducted a Phase I trial of cilengitide in malignant gliomas. The maximum-tolerated dose was not reached. The high dose of 2400 mg/m2 was well tolerated and there were two CRs, three PRs and four SD patients among 51 evaluable patients [107]. A Phase II trial of cilengitide is ongoing.

Proteases, such as MMPs, secreted by tumor cells are critical enzymes that degrade the extracellular matrix to facilitate cellular migration. MMPs also cleave numerous other enzymatic targets that may have opposing contributions to tumor growth and spread. MMP inhibitor monotherapy has limited efficacy in clinical trials, although the combination of MMP inhibitors with chemotherapy has demonstrated efficacy. A Phase II trial of marimastat plus temozolomide in patients with recurrent malignant gliomas showed a 6-month PFS of 39% [23]. Side effects of severe joint and tendon pain in more than half of the patients tested may limit the use of marimastat. Intracellular signaling pathways involved in the acquisition of resistance to apoptosis by migrating glioma cells are also important in the process of invasion. These pathways include phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB/Akt), mTOR, nuclear factor (NF)-κB and autophagy. Therefore, by targeting these pathways, tumor cell migration will be affected. Other potential targets in tumor cell invasion include plasminogen activator inhibitor (PAI)-1, Src kinase, cathepsin-B, urokinase-type plasminogen activator receptor (uPAR), SPARC, focal adhesion kinase (FAK), integrin-linked kinase (ILK), proline-rich tyrosine kinase 2 (Pyk2), ephrin receptor (Eph)B2 and RAC-Rho family GTPases are under preclinical investigation [108–113].

### Apoptosis-promoting or -modulating agents

Apoptosis, commonly called programmed cell death, is a major mechanism of cell death in response to many toxic stimuli, including withdrawal of external survival signals and DNA damage. In addition, cancer cells can die or permanently cease proliferation in nonapoptotic pathways, such as necrosis, senescence, autophagy and mitotic catastrophe [114]. Resistance to apoptosis is one of the hallmarks of cancer. Apoptosis is mediated by a family of cysteine proteases known as the caspases. There are two pathways by which caspase activation is triggered: the extrinsic and intrinsic apoptotic pathways. The extrinsic pathway is activated by the engagement of death signals (FAS ligand or tissue...

### Box 1. Summary of targeted therapeutic agents in clinical trials of malignant gliomas. (cont.)

- **Fenretinide**
- **Arsenic trioxide**
- **Lonidamine**
- **Bortezomib (Velcade®; proteosome inhibitor)**

**Cytokine-conjugated toxins**

- IL-13-PE38QQR (cilengitide besudotox; IL-13 conjugated pseudomonos toxin)
- TP-38 (TGF-α conjugated with pseudomonos toxin)

**Radiolabeled monoclonal antibodies**

- 81C6 (α1-3I-conjugated antibody to tenasin)

**EGFR: Epidermal growth factor receptor; HDAC: Histone deacetylase; Hsp: Heat-shock protein; IL: Interleukin; mTOR: Mammalian target of rapamycin; MMP: Matrix metalloproteinase; PDGFR: Platelet-derived growth factor receptor; PI3K: Phosphatidylinositol 3-kinase; PKC: Protein kinase C; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; VEGFR: Vascular endothelial growth factor.**

### Box 2. Potential new targeted therapies in preclinical and early clinical development.

**Cell cycle regulators**

- Aurora kinase inhibitors: MLN8054, AZD1152 and VX-680
- Polo-like kinase inhibitors: BI 2536
- Mitotic kinesin inhibitors: Ispinesib (SB-715992)
- Cyclin-dependent kinase inhibitors: flavopiridol

**Angiogenesis inhibitors**

- HIF-1α inhibitors: 2-methoxyestradiol (2ME2; Panzem®) and PX-478
- Pan-VEGFR inhibitors: GW786034 and AZD2171
- VEGF-soluble decoy receptor: VEGF-Trap
- VEGFR and PDGFR inhibitor: AMG-706
- VEGFR, FGFR, FLT-3 and PDGFR inhibitor: CHIR-258
- VEGFR, FGFR and PDGFR inhibitor: BIBF1120
- Angiopoietin-2 inhibitor: AMG-386 peptibody

**Apoptosis-modulating agents**

- Hsp70 inducer: STA-4783
- Poly (ADP-ribose) polymerase (PARP) inhibitor: AG14361, AG14699, INO-1001 and INO-1002
- Proteosome inhibitor: NPI-0052

**Agent targeting invasion**

- Src/Abl kinase inhibitor: dasatinib (BMS-354825)
- Anti-hepatocyte growth factor neutralizing antibody (Amgen)

**Multitargeted kinase inhibitors**

- CDKs, VEGFR and PDGFR inhibitor: ZK304709
- Mitosis and angiogenesis inhibitor: RO0281501
- EGFR and HER2 inhibitor: BIBW 2992

**CDK: Cyclin-dependent kinase; EGFR: Epidermal growth factor receptor; Hsp: Heat-shock protein; PDGFR: Platelet-derived growth factor receptor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor.**
necrosis factor (TNF) and cell surface death receptors (FAS or TNF-receptor). The intrinsic pathway is triggered by various extracellular and intracellular stresses, such as growth factor withdrawal, hypoxia, DNA damage and oncogene induction. Signals that are transduced in response to these stresses converge on the mitochondria. A series of biochemical events result in the permeabilization of the outer mitochondrial membrane, the release of cytochrome c and other proapoptotic molecules and the formation of the apoptosome. Among these processes, only the permeabilization step is regulated, where antiapoptotic members of the family can halt apoptotic death [115]. Cell death is also modified by other mitochondrial proteins. Direct IAP binding protein with low pI (DIABLO, also known as SMAC) promotes caspase activation by counteracting inhibitor of apoptosis (IAP)-mediated caspase inhibition [116]. Both intrinsic and extrinsic pathways converge to a final common pathway involving activation of caspases that cleave several structural molecules leading to cell death. Owing to this complex interaction of several proteins regulating apoptosis, selective inhibition of single pathways may not be effective to promote apoptosis in cancers. Although they are not originally described as proapoptotic agents, several drugs induce apoptosis through RAS/RAF/M EK/ERK or PI3K/Akt pathways. Several therapeutic agents directly targeting apoptotic machinery have been developed.

Agents that target extrinsic apoptosis pathways include recombinant human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and all-trans retinoic acid (ATRA). The efficacy of TRAIL for the treatment of glioma was limited owing to cellular resistance and, importantly, severe toxicity and poor distribution after systemic administration [117]. Recent preclinical data demonstrated that convection-enhanced delivery (CED) of TRAIL plus systemic administration of temozolomide prolonged survival of animals with intracranial glioma xenografts [118]. Retinoic acid has antitumor effects in an animal model of glioma [119]. The Radiation Therapy Oncology Group (RTOG) conducted a Phase II study of AT RA in recurrent GBM, demonstrating only modest efficacy [120]. Feretnide (4-hydroxyphenylretinamide), a synthetic retinoid, induced apoptosis of malignant gliomas in preclinical studies, but was ineffective in patients with recurrent malignant gliomas in a Phase II clinical study [121].

Agents that target the intrinsic apoptosis pathways

Arsenic trioxide is FDA-approved for the treatment of acute promyelocytic leukemia [122]. Arsenic trioxide induces caspase-independent autophagic cell death in malignant glioma cell lines through upregulation of the mitochondrial cell death protein, Bcl-2 adenovirus E1B 19kDa interacting protein (BNIP3) [123,124]. Lonidamine is a derivative of indazole-3-carboxylic acid that acts on the mitochondria to induce apoptosis through the disruption of the intrinsic transmembrane potential. It has a potential antiproliferative effect by inhibiting oxygen consumption and interfering with the energy metabolism of cancer cells. Cellular metabolism in GBM is characterized by a high rate of aerobic glycolysis that is dependent on mitochondria-bound hexokinase. Moreover, high levels of glucose utilization and tumor aggressiveness in GBM are associated with a high density of mitochondrial benzodiazepine receptors. Although the combination of lonidamine with diazepam demonstrated preclinical in vitro and in vivo activities against glioblastoma, a Phase II study of this combination failed to demonstrate a clinical response [125,126]. Bcl-2 and Bcl-XL share high sequence homology regions but inhibit apoptosis through different mechanisms. Several antisense oligonucleotides to these molecules have been developed but may be limited in efficacy owing to delivery limitations. Several small molecule inhibitors of Bcl-2 and Bcl-XL are under development. The synthetic retinoid N-(4-hydroxyphenyl) retinamide in combination with BCNU markedly downregulated levels of Bcl-XL and Bcl-2 proteins in glioblastoma [127]. This finding may serve as a foundation to sensitize gliomas to chemotherapy by targeting these antiapoptotic proteins.

Agents that target the common apoptotic pathways (caspase activation)

Survivin is a member of the IAP family that regulates cell division and suppresses apoptosis. The precise role of survivin in tumor behavior remains unclear; however it may prevent apoptosis through the inhibition of caspases [128]. Survivin expression was inversely correlated with apoptotic index in human glioma samples [128]. Several inhibitors of IAP and survivin have been developed and are under investigation in preclinical studies [129].

Other agents that modulate the apoptosis pathways include proteasome inhibitors, NFκB inhibitors and mTOR inhibitors. The ubiquitin–proteasome system is important in regulating the intracellular level of proteins, hence balancing cell proliferation and apoptosis [130]. Bortezomib (Velcade®; PS-341; Millenium Pharmaceuticals, MA, USA), a proteasome inhibitor, induced growth arrest and apoptosis in glioma cell lines and explants [131]. A Phase I/II study in malignant gliomas is ongoing.

mTOR inhibitors

mTOR (also known as FK-506-binding protein 12-rapamycin-associated protein 1) is a downstream component of the PI3K/Akt pathway that regulates proliferation by activating downstream protein kinases required for ribosomal biosynthesis and mRNA translation. mTOR activates the 40S ribosomal protein S6 kinase (p70S6K) and inhibits the initiation factor 4E-binding protein (4E-BP)-1, driving the cell cycle progression from G1 to S phase. Tumors that depend on the activation of the PI3K pathway, or that harbor mutations causing constitutive activation of the PI3K pathway, may be more susceptible to rapamycin and derivatives that inhibit mTOR. PTEN-deficient cancer cell lines, which exhibited a constitutively active PI3K pathway, have been shown to increase the sensitivity to an mTOR inhibitor [132]. Activation of the PI3K pathway by overexpression of upstream growth factor receptors, such as EGFR, and/or deletion of PTEN, is significantly associated with increasing tumor grade, decreased levels of apoptosis and adverse clinical outcome in human gliomas [133]. Therefore, mTOR would appear to be a promising target in the clinical management of glioma patients. Several mTOR inhibitors have been developed in clinical trials for malignant gliomas.
including rapamycin (Rapamune®; sirolimus; Wyeth, PA, USA), CCI-779 (temsirolimus; Wyeth, PA, USA), RAD001 (everolimus; Novartis, NJ) and AP23573 (Ariad pharmaceuticals, MA, USA). Temsirolimus has shown preclinical activities against human glioma cell lines [134]. A Phase II study of temsirolimus in recurrent GBM’s revealed 36% radiographic improvement, as determined by a decrease in T2 signal abnormalities and/or a decrease in gadolinium-enhanced T1 weighted lesions. The 6-month PFS was 7.8% and median time-to-progression was 2.3 months. Significant correlation was observed between radiographic improvement and high levels of phosphorylated p70S6K in baseline tumor samples [135]. Another Phase I/II trial of 29 patients by the NABTC showed 7% PR and 41% SD patients without prolonged 6-month PFS [136]. These studies suggested that a single-agent mTOR inhibitor has minimal activity against malignant gliomas. Future trials may need to better stratify patients by incorporating the PTEN and P70 S6K status. In addition, the combination of mTOR inhibitors with chemotherapy and other small molecule inhibitors should be explored. Recently, the author’s group demonstrated that a combination of EGFR and KDR inhibitor (AEE788) and mTOR inhibitor (RAD001) displayed increased rates of cell cycle arrest and apoptosis and reduced proliferation compared with either agent alone. In vivo studies confirmed greater tumor growth inhibition and greater increases in median survival than monotherapy [92]. Another study has also demonstrated a combinatorial benefit of this similar approach in a glioblastoma model by using an EGFR kinase inhibitor, EKI-785, and an mTOR inhibitor, rapamycin [137]. Several clinical studies based on this rationale of combination by targeting both upstream growth factor receptor signaling pathways and the downstream PI3K pathway are ongoing. The NABTC is conducting a Phase I/II study of erlotinib plus temsirolimus, whereas other groups are conducting combinations of gefitinib plus rapamycin and AEE788 plus RAD001 [138]. Other combinations, such as sorafenib plus temsirolimus, are planned. Lastly, several inhibitors of the PI3K/Akt pathway have been developed. Perifosine (KRX-0401; Keryx Biopharmaceuticals, NY, USA), an oral Akt/AMPK inhibitor, has demonstrated promising preclinical activity when combined with temozolomide in a transgenic glioma model [139]. Perifosine is now under clinical trial development in malignant gliomas.

**Farnesyltransferase inhibitors**

Signal transduction from activated receptor tyrosine kinases are partly mediated through the RAS/RAF/M EK/ERK pathway. Malignant gliomas exhibit activity of three major types of RAS: N-RAS, H-RAS and K-RAS [140]. Although all three RAS members can be farnesylated, K-RAS, which is the most frequently mutated form in human cancers, can also be geranylgeranylated. In preclinical studies, broad spectrum inhibitors of farnesyltransferases and geranylgeranyltransferases were toxic. Specific inhibitors of farnesyltransferases can block the mitogenic function of RAS but also block the post-translational modification of many farnesylated proteins, such as RhoB, RhoE, the nuclear lamins and Rap-2 [141]. Farnesyltransferase inhibitors (FTIs) have activity against human gliomas and two specific FTIs (tipifarnib, lonafarnib [SCH 66336; Schering-Plough, NJ, USA]) have been developed [142]. The NABTC completed a Phase I/II study of tipifarnib in recurrent malignant gliomas. There were significant differences of pharmacokinetic parameters between patients taking enzyme-inducing anti-epileptic drugs (EIAEDs) and patients not taking EIAEDs [143]. In the Phase II part of the study of patients not receiving EIAEDs, the 6-month PFS rate was 24% with only three PR patients, suggesting a modest efficacy of FTI as a monotherapy in recurrent malignant gliomas. Studies combining FTIs with radiation therapy, temozolomide and other targeted therapies are under development.

**Protein kinase C inhibitors**

For decades, tamoxifen has been used in breast cancers as an estrogen modulator. Tamoxifen inhibits protein kinase C (PKC), insulin-like growth factor (IGF)-II and N FκB pathways in glioma cell lines and has activity against glioma xenografts in vivo [144,145]. The true molecular target of tamoxifen remains controversial. Several studies have shown modest efficacy of tamoxifen in recurrent malignant gliomas [146,147]. Tamoxifen has been shown to sensitize glioma cell lines to chemotherapy agents, such as irinotecan [148]; however, several Phase II studies of tamoxifen plus procarbazine, temozolomide or BCNU failed to demonstrate clinical or radiographic responses [149–151]. Given the disappointing clinical trial results, a strategy to improve the cytotoxicity of tamoxifen has been developed. IGF-1 is a naturally occurring, thyroid hormone-related polypeptide. Monoclonal antibodies against tamoxifen-induced cytotoxicity. Chemical hypothyroidism was induced in 22 malignant glioma patients receiving high-dose tamoxifen. Median survival was significantly longer in the hypothyroid patients than in the euthyroid patients [152]. Further clinical studies are needed to confirm the efficacy.

Activation of PKCβ has been implicated in tumor-induced angiogenesis, tumor cell proliferation, apoptosis and tumor invasiveness. The PKCβ-selective inhibitor, enzastaurin (LY317615; Eli Lilly, IN, USA), demonstrated preclinical activity against glioblastoma xenograft [153] and is being tested in a clinical trial for recurrent malignant gliomas, with promising activity [154]. Another PKC, the epsilon subtype (PKCε), may also play an important role in apoptosis. Downregulation of PKCε by siRNA enhanced the apoptotic effect of TRAIL in glioma cell lines. Moreover, PKCε regulates Akt expression and is essential for the survival of glioma cells [155]. Development of a selective inhibitor of PKCε may be warranted.

**Cyclooxygenase-2 inhibitors**

High cyclooxygenase (COX)-2 expression was associated with increasing histological grade and poor survival outcome in gliomas [156]. Celecoxib (Celebrex®; Pfizer, NY, USA), a selective COX-2 inhibitor, has antitumor activity in a rat intracranial glioma model [157]. The authors’ group recently reported a Phase II study result of irinotecan plus celecoxib 400 mg twice daily in patients with recurrent malignant gliomas [158]. A total of 16% of patients with GBM had radiographic response and 35% achieved SD. The median PFS was 11.0 weeks and the 6-month
PFS was 25.1% [158]. Given the recent report of celecoxib associated with cardiovascular death in a colorectal cancer prevention trial [159], the enthusiasm for celecoxib use as a therapeutic agent in malignant glioma may be decreased.

**Deacetylase inhibitors**

The histone code refers to specific modifications of nucleosome-associated histone proteins involved in the regulation of gene transcription. These modifications include acetylation, methylation, and phosphorylation of several histone amino acid residues and are associated with different states of chromatin configuration and gene expression. In particular, acetylation of specific residues in histones has been associated with an open chromatin configuration and a permissive gene transcription state. This particular modification is regulated by several enzymatic activities with the capacity to either transfer acetyl groups (histone acetyltransferases [HATs]) or to induce histone deacetylation (histone deacetylases [HDACs]). This last activity is associated with gene silencing [160]. The HDAC family includes deacetylases (HDACs 4, 6 and 10) that function to regulate key cytoplasmic targets to regulate growth factor degradation, suggesting that nonhistone targets may also contribute to the efficacy of deacetylase inhibitors [161]. Inhibition of HDAC has been associated with cell differentiation and induction of apoptosis in malignant glioma. Pretreatment with a HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), can sensitize glioma cells to radiation and chemotherapy [162–164]. This agent, and other HDAC inhibitors, such as depsipeptide (FK228; Gloucester Pharmaceuticals, MA, USA) and valproic acid, are under clinical development in malignant gliomas.

**Chaperone protein inhibitors**

Heat-shock protein (Hsp)90 is an ATP-dependent molecular chaperone that functions to stabilize regulatory proteins through the formation of functional hetero-oligomeric complexes [165]. Many of the client proteins are involved in cell cycle regulation and cell survival and are associated with cytoprotection against DNA damage. Therefore, targeting this chaperone protein function may have a direct antineoplastic effect or may enhance cytotoxicity of radiation and chemotherapy to cancer cells. Geldanamycin was shown to inhibit the proliferation of glioma cell lines in a preclinical study [166]. In addition, 17-allylamino-17-demethoxygeldanamycin (17-AAG), a less toxic and less potent derivative of geldanamycin, significantly inhibited the growth of glioma xenografts in nude mice [166]. Recent data also showed enhanced tumor cell radiosensitivity by another orally-available Hsp90 inhibitor, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) [167]. Clinical development of Hsp90 inhibitors in malignant gliomas appears warranted.

**TGF-β inhibitors**

TGF-β is a multifunctional cytokine expressed in malignant gliomas that increases tumor cell motility, invasion, angiogenesis and immune escape [168]. Malignant glioma cells secrete TGF-β ligands and express receptors, indicating the presence of an autocrine loop. Preclinical studies have shown promise using antisense oligonucleotides directed to mRNAs encoding TGF-β ligands. AP12009 (Antisense Pharma, Germany), a TGF-β ligand-specific antisense oligonucleotide, was well tolerated when administered intratumorally. Median survival for GBM was 47 weeks [169]. Several small molecule ATP mimetics of TGF-β receptors have shown efficacy in preclinical studies of malignant gliomas [170,171]. These agents might have utility either as monotherapies or in combination with chemotherapy or is immunomodulatory treatment.

**Combination therapy**

Several potential resistance mechanisms to molecularly targeted therapeutic agents have become apparent. Cell lines treated with growth factor receptor inhibitors usually regrow with increased expression of other mitogenic growth factors. Preclinical studies of glioma cell lines that are resistant to EGFR kinase inhibitors exhibited activation of the IGF1/PI3K/Akt pathway [172,173]. Therefore, disrupting several growth factor pathways by multi-targeted kinase inhibitors, or by the combination of several inhibitors to disrupt multiple mitogenic pathways, might offer greater benefit. For example, targeting both EGFR/VEGFR and mTOR pathways by AEE788 and RAD001 has demonstrated combinatorial benefits in a preclinical study from the authors' group [92]. Based on this rationale, several clinical trials in malignant gliomas are ongoing, including erlotinib plus temsirolimus, gefitinib plus rapamycin and AEE788 plus RAD001. Sequential administration of each drug is important to avoid negative interactions. For instance, some agents might require cells to be cycling to induce apoptosis, whereas others might only induce cell cycle arrest.

A recent study showed synergistic antitumor activity of the PI3K inhibitor LY294002 and the Hsp90 inhibitor 17-AAG in malignant glioma cell lines [174]. An in vivo study of this combinatorial approach may be required prior to clinical development. Multimodality treatment with conventional radiation or stereotactic radiosurgery should also be explored. Several preclinical studies demonstrate that small molecule inhibitors can sensitize tumors to radiation or can have synergistic antitumor activity with radiation therapy [175–177]. Careful design of the clinical trials of such multimodality treatments is important. Sequencing and timing of drug administration in relation to radiation treatment is crucial as there are significant differences on combinatorial benefits in an animal study [176,178].

**Expert commentary & five-year view**

Current targeted therapies largely focus on the disruption of oncogenic signaling pathways, either growth factor receptors or intracellular effectors. The success of imatinib mesylate in chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor (GIST) has stimulated the use of targeted therapies in solid cancers, including malignant gliomas. Using targeted therapies in malignant glioma is not as simplistic as in CML, where a single dominant oncogenic mutation is present and is ‘druggable’ by imatinib mesylate. No subset of malignant gliomas has been found to be dependent on a single oncogene or tumor-suppressor gene. Most malignant gliomas show a wide array of genetic
changes consistent with genomic instability. Therefore, it is unlikely that targeting one oncogenic pathway will be sufficient to control tumor growth in a wide range of patients. Most clinical trials of single-targeted agent monotherapy, such as EGFR, VEGFR, PDGFR or mTOR inhibitors, in recurrent malignant gliomas have failed to demonstrate survival benefits.

Several mechanisms of resistance to these new therapies must be addressed. Although some small molecule inhibitors may be able to penetrate the blood–brain barrier, they may not be able to reach the tumor cells due to high tumor interstitial pressure or active efflux of the drugs by multidrug transporters, such as P-glycoprotein and BCRP (ABCG2). Decreasing tumor interstitial pressure by imatinib mesylate improves drug delivery to the tumor [179]. Modulating multidrug transporter proteins also increases brain penetration of small molecule inhibitors [180]. In addition, several small molecule ATP mimetics also sensitize tumors to chemotherapies, such as irinotecan, possibly by down-regulating the ABCG2 activity [72]. Further preclinical and clinical studies to improve drug delivery are required. Another major mechanism of intrinsic resistance of tumor to targeted therapies is the ability of cancer cells to escape through the alternative mitogenic pathways. Therefore, targeting multiple growth pathways both upstream (receptors) and downstream (intracellular effectors) by using either multikinase inhibitors or a combination of several single-kinase inhibitors may overcome the resistance. Recent encouraging activity of bevacizumab and irinotecan confirm the important role of antiangiogenic therapy in addition to targeting tumor cells by chemotherapy. Future clinical trials should consider vascular disrupting agents as a potential component of combination therapy. Several new biological therapies, such as oncolytic viruses, gene therapy and immunotherapy, will also be employed. New technology for delivery of small molecule inhibitors or biological markers, such as nanoparticle delivery systems, will become more available. New imaging techniques, such as dynamic contrast enhanced (DCE) MRI, will be more widely studied as a pharmacodynamic measure of antiangiogenic therapy. New positron emission tomography (PET) techniques, such as 18-Fluorothymidine-PET (FLT-PET), may be used to distinguish proliferating tumor from radiation- or chemotherapy-induced cerebral necrosis. In vivo imaging of signal transduction pathways in the tumor may become available in the very near future.

Finally, the role of cancer stem cells in gliomagenesis and resistance to radiation and chemotherapy will undergo extensive evaluation. Recent isolation of cancer stem cells has shifted the paradigm of research in neuro-oncology [181]. These glioma stem-like cancer cells also express the surface marker CD133, in addition to conventional markers for neural stem cells. These cells form the tumors recapitulating parental histopathology when xenotransplanted into the mouse brain. As the molecular abnormalities of glioma stem-like cancer cells are examined, targeted therapies aimed at these cells will be developed simultaneously. Targeting cancer stem and non-stem cells may offer a new horizon in the treatment of this devastating tumor.

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Key issues

- Recent understanding of the molecular pathogenesis of malignant gliomas has provided an opportunity to develop targeted therapies against aberrant signal transduction pathways.
- Identification of molecular markers that correlate with the clinical response of each targeted therapy in malignant gliomas will become essential to design better clinical trials.
- Sequential assessment of drug levels (pharmacokinetics), delivery, target inhibition (pharmacodynamics) and biological efficacy must be incorporated in development paradigms of targeted therapies. Tissue acquisition will be essential in preclinical and clinical trials.
- Brain tumor patients frequently require increased doses of targeted therapeutic agents owing to the common use of hepatic cytochrome P450 -inducing agents, such as some antiepileptics and steroids, and altered volumes of distribution.
- Combination of several molecularly targeted therapeutic agents with radiation, chemotherapy and immunotherapy will undergo clinical evaluation. Novel inhibitors targeting multiple molecular targets with reduced toxicities will be developed.
- Encouraging preliminary clinical data of bevacizumab confirmed the pivotal role of anti-angiogenesis in glioma therapy and has stimulated the plan of future clinical trials of bevacizumab or other vascular disrupting agents plus radiation, other chemotherapies or molecularly targeted therapies.
- Recent discovery of cancer stem cells from tumors of patients with malignant glioma has stimulated a new area of research in neuro-oncology. As the molecular abnormalities of glioma stem cells are being elucidated, these cells will become attractive targets for treatment.
References

Papers of special note have been highlighted as:

* of interest
** of considerable interest


** Pivotal Phase III trial demonstrating a survival benefit of adjuvant temozolomide plus radiation therapy in patients with glioblastoma.


• Large Phase III trial of 1,3-bis(2-chloroethyl)-1-nitrosurea (BCNU)-wafers in the treatment of malignant gliomas.


• Excellent review of cancer biology.


• First report on the outcome of a small molecule epidermal growth factor receptor (EGFR) kinase inhibitor in patients with recurrent glioblastoma.


• Elegant study defining molecular markers of clinical response for glioblastomas to EGFR kinase inhibitors.

New treatment strategies for malignant gliomas


Rich JN, Sathornsumetee S, Keir ST et al. ZD 6474, a novel tyrosine kinase inhibitor of vascular endothelial growth factor receptor and epidermal growth factor receptor.


New treatment strategies for malignant gliomas


 Recent review on the ubiquitin–proteasome system in cancer.


Kawaguchi Y, Kovacs JJ, LaLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation


Kawaguchi Y, Kovacs JJ, LaLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation


• Recent identification of glioma cancer stem cells that has stimulated the paradigm shift in neuro-oncology research in both cancer biology and therapeutic discovery.

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