New approaches to primary brain tumor treatment

Sith Sathornsumetee[a,b] and Jeremy N. Rich[a,b,c]

Primary brain tumors represent over 100 different tumor types with widely divergent biologies and clinical outcomes, but these neoplasms frequently pose similar challenges to neuro-oncologists. Malignant gliomas are the most common type of primary intrinsic brain tumor in adults and remain extremely lethal. Current standard-of-care therapies for these cancers include surgery, radiation and palliative cytotoxics, which have significant side-effects and limited efficacy. Advances in our understanding of the molecular underpinnings of cancer have led to targeted molecular therapies that may permit improvement in therapeutic efficacy and reduced toxicity; these therapies, however, still face many challenges. Signal transduction pathways that are inappropriately regulated in brain cancers include growth factors and their receptors (e.g. epidermal growth factor receptor, vascular endothelial growth factor receptor and platelet-derived growth factor receptor), which regulate cellular interactions with the microenvironment and intracellular oncogenic pathways. Low-molecular-weight inhibitors have been developed to target many kinases and may have advantages in terms of delivery. Monoclonal antibodies may have greater specificity, but face delivery restrictions. Preferential tumor delivery of chemotherapies, conjugated toxins and radioisotopes has been achieved through convection-enhanced delivery, intratumoral implants and intra-arterial infusion. Despite these advances, few molecularly targeted therapies have demonstrated significant antineoplastic activity for a broad range of patients, possibly due to tumor and patient heterogeneity. Improved functional neuropathology and imaging may permit identification of patient subgroups for which clinical responses may be enriched. It is probable, however, that targeted therapies will be most effective in combination either with one another or with cytotoxic therapies. In this study, we review the current state of new therapies for malignant gliomas. *Anti-Cancer Drugs* 17:1003–1016 © 2006 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2006, **17:**1003–1016

Keywords: brain cancer, chemotherapy, glioma, kinase inhibitor, targeted therapy

Departments of [a]Medicine, [b]Surgery and [c]Neurobiology, The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, North Carolina, USA.

Correspondence to J. N. Rich, Departments of Medicine, Surgery and Neurobiology, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, DUMC 2900, Durham, NC 27710, USA.
Tel: +1 919 681 1693; fax: +1 919 684 6514;
e-mail: rich0001@mc.duke.edu

Sponsorship: This work was supported in part by funds from the Childhood Brain Tumor Foundation, Accelerate Brain Cancer Cure and the Pediatric Brain Tumor Foundation of the United States (J.N.R.). This work was also supported by NIH grants NS047409, NS054276 and 1 P50 CA108786 (J.N.R.). J.N.R. is a Damon Runyon–Lilly Clinical Investigator supported by the Damon Runyon Cancer Research Foundation and a Sidney Kimmel Cancer Foundation Scholar.

Note: Due to the breadth of the area under review, it was not possible to include many excellent studies conducted by our colleagues in neuro-oncology in the space allotted. We regret the omissions.

Received 17 April 2006 **Accepted** 5 June 2006

Introduction

The incidence of all primary non-malignant and malignant brain and central nervous system tumors is 14.8 cases per 100 000 person-years [1]. The worldwide incidence rate of primary malignant central nervous system tumors is 3.7 cases per 100 000 person-years for males and 2.6 cases per 100 000 person-years for females [1]. An estimate of 18 500 new cases of primary malignant central nervous system tumors was diagnosed in the US in 2005. This estimate represented 1.35% of all cancers in the US. Although primary central nervous system tumors are relatively uncommon, they are highly lethal with an estimate of 12 760 deaths in 2005 in the US [1]. Malignant gliomas are the most common primary central nervous system tumors in adults. The incidence of malignant gliomas in the US 5–10 per 100 000 persons with more than 15 000 new cases each year [1]. Despite recent progress in cancer research and treatment, prognosis remains poor with a median survival of 10–12 months for glioblastoma multiforme (GBM), the most common form of malignant gliomas. Recent advances in the understanding of molecular abnormalities and mechanism of resistance to radiation and chemotherapy in malignant gliomas have provided new approaches to target specific aberrant signal transduction pathways. Several small-molecule inhibitors and monoclonal antibodies have been developed in preclinical and clinical studies. Targeting the underlying pathogenesis may offer an innovative therapeutic approach for patients with these devastating tumors. Here, we review the new therapeutic strategies for malignant gliomas with an emphasis on molecularly targeted therapy.

Gliomas are named on the basis of histologic similarity to glial cells, including astrocytes, oligodendrocytes and ependymal cells. Astrocytomas are the most common glial

0959-4973

tumors. The World Health Organization (WHO) classified astrocytomas into four grades according to histological appearance [2,3]. Pilocytic astrocytomas (WHO grade I) are relatively benign and more common in children and young adults. Surgical resection of the tumor, if accessible, is a standard treatment and may be curative. External beam radiation therapy and chemotherapy may be effective in symptomatic, unresectable cases. Many patients survive for decades after diagnosis. Low-grade or diffuse astrocytomas (WHO grade II) are slow-growing, but infiltrative tumors, frequently diagnosed in young adults, with seizures as a common presenting symptom. Survival with low-grade gliomas is variable, with a median of 5–10 years, and most patients die from progression with transformation to higher-grade tumors [4]. Management of low-grade astrocytoma is highly controversial, with approaches ranging from observation in asymptomatic cases to surgery, chemotherapy and/or radiation with progressive tumors. Malignant or high-grade gliomas include anaplastic gliomas (anaplastic astrocytoma, anaplastic oligodendroglioma and anaplastic mixed oligoastrocytoma), designated as WHO grade III, and GBM and gliosarcoma designated as WHO grade IV. Histopathological characteristics of malignant gliomas are mitoses and cellular/nuclear atypia, and, in glioblastomas, vascular proliferation and necrosis with pseudopalisading features [5]. The prognosis of patients with malignant gliomas remains dismal; the median survival of anaplastic astrocytoma patients is 2–3 years and that of GBM patients is 10–12 months [6]. The prognosis of patients with oligodendroglioma is better than that of those with astrocytoma, particularly in oligodendroglioma patients with loss of chromosomes 1p and 19q, which are associated with sensitivity to chemoradiotherapy [7]. Favorable prognostic factors include young age, good performance status and maximal surgical resection of the tumor [8,9]. Current therapies for patients with malignant gliomas remain marginally effective. Recurrence following surgery, radiation therapy and adjuvant chemotherapy is nearly universal. Available salvage chemotherapies following progression are ineffective, with a 6-month progression-free survival of 15% and median progression-free survival of only 9 weeks among 225 patients with recurrent GBM [8]. Novel strategies targeting molecular aberrations may offer innovative therapeutic approaches for patients with these devastating tumors.

Current standard treatment

Most high-grade glioma patients usually undergo multimodality treatments, including surgical resection, radiation and chemotherapy, after histologic diagnosis. Although there are conflicting data, maximal surgical resection of tumors significantly improves survival in some studies [10]. Radiation therapy has been the main standard-of-care treatment following biopsy or resection with minimal utility of systematically administered nitrosoureas until recently when Stupp *et al.* [11] and Taphoorn *et al.* [12] reported that adjunctive chemotherapy (concurrent daily temozolomide with radiation, followed by 6 months of monthly temozolomide) increases median survival by 2.5 months without degradation in quality-of-life and significantly increases 2-year survival rate. Although the survival benefits of adjuvant chemotherapy are modest compared with those seen in other solid cancers, these findings suggest that novel therapies may improve patient outcomes without severe side-effects.

Salvage chemotherapy

Temozolomide

Temozolomide (Temodar; Schering-Plough, Kenilworth, New Jersey, USA) is an orally available methylating agent that has become the first-line chemotherapy for malignant glioma. The response rate, however, is modest and of limited duration. In a study of temozolomide in patients with recurrent anaplastic gliomas (WHO grade III), 8% of patients had a complete response, 27% partial response and 27% stable disease, with 46% of patients having progression-free survival of at least 6 months [13]. For patients with recurrent GBM, there was a 5% partial response rate and a 40% stable disease rate, with a 6-month progression-free survival of only 21% [14]. Several studies have identified a potential biomarker of clinical response to temozolomide. O^6-alkylguanine-DNA alkyltransferase [AGT; also known as MGMT (O^6-methylguanine-DNA methyltransferase)] is a DNA-repair protein that removes alkyl groups from the O^6 position of guanine, an important site of DNA alkylation [15]. MGMT is commonly expressed at low levels in endogenous tissues. Epigenetic silencing of the MGMT DNA-repair gene by promoter methylation has been associated with longer survival in patients with glioblastoma who receive temozolomide [16]. Therefore, targeting MGMT pharmacologically may represent a new way to overcome resistance to alkylating chemotherapy in malignant gliomas. O^6-benzylguanine (O^6-BG) is an MGMT substrate that inhibits MGMT by suicide inactivation. Preclinical studies suggest the enhancement of chemotherapy efficacy by O^6-BG in a murine model of gliomas [17,18]. Several phase I trials of intravenous O^6-BG alone [19] or in combination with alkylating agents, including carmustine [20] and temozolomide [21], in patients with malignant gliomas revealed that MGMT was depleted following intravenous administration of O^6-BG. A phase II trial of carmustine plus O^6-BG, however, failed to show clinical response and more than 50% of patients developed grade 3–5 hematologic toxicities [22]. A phase II trial of O^6-BG in combination with temozolomide has been completed.

Several clinical trials have demonstrated modest activity of temozolomide in combination with other chemotherapies such as procarbazine [23], biodegradable carmustine

(BCNU) [24], irinotecan [25,26] and etoposide [27]. Combinations of temozolomide with molecularly targeted therapies such as 13-*cis*-retinoic acid [28], marimastat [matrix metalloproteinase (MMP) inhibitor] [29] imatinib mesylate (Gleevec; Novartis, East Hanover, New Jersey, USA) [30], erlotinib (Tarceva; Genentech, South San Francisco, California, USA) [31] and gefitinib (Iressa; AstraZeneca, Wilmington, Delaware, USA) [32] have also been evaluated. The results of these trials, although preliminary, showed modest increase in efficacy when compared with temozolomide monotherapy.

Irinotecan

Irinotecan (Camptosar, CPT-11; Pfizer, New York, New York, USA) is a camptothecin derivative that acts as a prodrug that undergoes hydrolysis to active metabolite SN-38, a potent topoisomerase I inhibitor [33]. Irinotecan displayed robust antitumor activity against human glioma xenografts [34]; it also demonstrated encouraging clinical activity in an early clinical trial [35]. Several phase II trials from the New Approaches to Brain Tumor Therapy (NABTT), the North Central Cancer Treatment Group (NCTCG) and the North American Brain Tumor Consortium (NABTC), however, did not show survival benefits in patients with recurrent malignant glioma [36]. A combination of temozolomide plus irinotecan in phase I and II trials showed acceptable toxicity and encouraging antitumor activity with a 6-month progression-free survival rate of 29% [25,26].

Intra-arterial chemotherapy

The blood–brain barrier presents limitations for chemotherapy delivery to brain tumors when administered systemically. Although gliomas contain areas of disrupted blood–brain barrier integrity, areas of tumor invasion are protected to a varying degree. Direct tumor infusion of chemotherapy via a 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 agents (e.g. mannitol) before intra-arterial chemotherapy infusion. These approaches may induce significant, although transient, neurologic symptoms such as cerebral edema. Several small phase I and II studies using intra-arterial chemotherapy have been completed, but no phase III study at present has demonstrated the benefit of intra-arterial chemotherapy over its intravenous counterpart [37]. Intra-arterial chemotherapy administration following blood–brain barrier disruption is not widely available as it requires highly trained professionals to perform the procedure in specialized centers.

Brachytherapy

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 90% of GBM recurrences at or adjacent to the original tumor [38]. Therefore, direct intra-tumoral or intra-cavitary administration of chemotherapy, conjugated biologic toxin, or radiolabeled monoclonal antibody may improve local tumor control in patients with malignant glioma.

Carmustine (BCNU) wafers

Carmustine (BCNU) wafers (Gliadel; MGI Pharma, Minneapolis, Minnesota, USA) are the first Food and Drug Administration-approved biodegradable wafers containing nitrosourea, carmustine, for the treatment of malignant glioma. A recent large phase III trial with 240 patients with newly diagnosed malignant glioma randomized to receive either carmustine or placebo wafers at the time of primary surgical resection followed by radiation therapy demonstrated a modest survival benefit with median survival of 13.9 months for the carmustine wafer-treated group and 11.6 months for the placebo-treated group [39]. Side-effects were comparable between the two groups except for significantly higher rates of cerebrospinal fluid leak and intracranial hypertension in the carmustine-wafers treated group. Similarly, in patients undergoing resection for recurrent GBM, placement of carmustine wafers only provides modest prolongation of survival [40]. Combination of carmustine wafers with other agents such as systemically administered O^6-BG is ongoing.

Conjugated toxin therapy

Malignant gliomas commonly overexpress several cell surface receptors that undergo ligand binding and internalization, permitting the specific delivery of radio isotopes or toxins to tumor cells. Interleukin (IL)-13 receptors are abundantly expressed on the surface of glioblastoma cells, but not in normal brain or endothelial cells [41]. A recombinant fusion protein composed of IL-13 and a mutated form of pseudomonas exotoxin (IL13-PE38QQR; cintredekin besudotox; NeoPharm, Waukegan, Illinois, USA) has demonstrated antitumor efficacy after intracerebral administration but not with systemic administration. The exotoxin is exquisitely potent, inducing cell death with a single internalized molecule. On the basis of 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 [42]. The conjugated cytotoxin is delivered by convection-enhanced delivery (CED). Increased tumor interstitial pressure limits intratumoral drug delivery from systemic vasculature, but CED takes advantage of the pressure gradient by infusing small volumes at high pressures over long periods to permit full tumor delivery of therapeutic agents into the resection cavity or tumor bed through a stereotactically placed catheter [42]. A randomized phase III trial of IL-13 cytotoxin delivered by CED versus BCNU wafer

(PRECISE trial) has completed enrollment. A phase I trial of IL-13 cytotoxin in patients with newly diagnosed GBM is ongoing. Other cytokine-conjugated toxin therapies include IL-4-conjugated pseudomonas toxin, which can also bind the IL-13 receptors [43], transforming growth factor-α conjugated with mutated pseudomonas toxin (TP-38) that binds epidermal growth factor receptors (EGFRs) [44], transferring–CRM107, a conjugate of transferrin and diphtheria toxin [45], and DAB389EGF, a fusion protein composed of the catalytic and translocation domains of diphtheria toxin fused via a His–Ala linker to the human epidermal growth factor (EGF) [46].

^{131}I-labeled monoclonal antibody against tenascin

Tenascin is commonly expressed in high-grade gliomas, but not in the normal brain. Preclinical studies of ^{131}I-labeled monoclonal antibody against tenascin (^{131}I-m81C6) demonstrated significant tumor growth delay and regression in athymic mice bearing subcutaneous human glioma xenografts, and prolongation of median survival of athymic rats bearing intracranial tumors [47,48]. In a phase II trial, 33 patients with newly diagnosed malignant gliomas underwent surgical resection with intracavitary ^{131}I-m81C6 administration, followed by conventional external beam radiotherapy and a year of alkylator-based chemotherapy. Median survival for patients with GBM was 79.4 weeks [49]. Twenty-seven percent of patients developed reversible hematologic toxicity and 15% developed histologically confirmed, treatment-related neurologic toxicity [49]. A randomized phase II trial of ^{131}I-m81C6 administration in newly diagnosed malignant glioma patients before radiation therapy with concurrent temozolomide is ongoing. A phase III multicenter trial with patient-specific dosing is planned. In addition, ^{131}I-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 anaplastic astrocytoma/anaplastic oligodendroglioma tumors were alive at 1 year. Median overall survival for patients with GBM and anaplastic astrocytoma/anaplastic oligodendroglioma tumors was 64 and 99 weeks, respectively [50]. Other radioactive brachytherapy treatments such as ^{125}I bead administration have also been used for localized recurrences of malignant glioma. Further clinical trial data are needed before routine use of these treatments.

GliaSite radiation therapy system

GliaSite radiation therapy system (GliaSite RTS; Cytyc, Atlanta, Georgia, USA) consists of a silicone spherical balloon catheter and an aqueous radiation source (Iotrex [sodium 3-(^{125}I)-iodo-4-hydroxybenzenesulfonate]). Two recent independent studies of GliaSite RTS in patients with recurrent GBM demonstrated median survival of approximately 36 weeks after the treatment [51,52]. The only prognostic factor to suggest favorable survival is a good Karnofsky performance status (KPS > 70). Treatment was generally well tolerated, although a few patients from each trial developed symptomatic radiation necrosis. Additional clinical trials are required to fully address the therapeutic efficacy of the GliaSite RTS.

Stereotactic radiotherapy

As stated above, current standard treatment for GBM following histologic diagnosis is external beam fractionated radiotherapy with concurrent temozolomide. Radiation therapy in newly diagnosed GBM provides unequivocal survival benefit; however, the role of stereotactic radiotherapy following recurrences remains unclear. The American Society for Therapeutic Radiology and Oncology (ASTRO) released an evidence-based review of the role of stereotactic radiosurgery (SRS) for malignant glioma in 2005 [53]. In this review, the authors concluded that there was insufficient evidence to support the use of SRS at the time of progression or recurrence. Insufficient evidence was also found regarding the benefits/harms in the use of stereotactic fractionated radiation therapy for patients with newly diagnosed or progressive/recurrent malignant glioma. Following the release of this review, several studies have been published to address the risks and benefits of SRS and stereotactic fractionated irradiation in recurrent malignant gliomas. A recent case-control study demonstrated similar survival advantage in patients with localized recurrent GBM who underwent either surgical resection or SRS [54]. Combs *et al.* [55] conducted a retrospective study of SRS in 32 patients with recurrent GBM. All patients initially received fractionated external beam radiation as a standard treatment following diagnosis. The median interval between primary irradiation and re-irradiation was 10 months. The median dose applied was 15 Gy. The 6-month progression-free survival was 72% and the 1-year progression-free survival was 28%. Survival benefit was more prominent in patients with smaller lesions at recurrence [55]. Another study from the same group evaluated the efficacy of fractionated stereotactic radiotherapy in patients with recurrent gliomas [56]. Forty-two patients with anaplastic gliomas and 59 patients with GBM were treated with fractionated stereotactic radiotherapy following recurrence. The median dose was 36 Gy delivered in a median fractionation of 5×2 Gy/week. Progression-free survival after fractionated stereotactic radiotherapy was 5 months for GBM and 8 months for anaplastic gliomas. Treatment was well tolerated. Prospective study is required to further evaluate the efficacy.

Molecular alteration in malignant gliomas

Malignant gliomas, like other cancers, share common, essential characteristics – self-initiated proliferation, avoidance of apoptosis, evasion from immune surveillance, new blood vessel formation and an ability to invade

normal tissues [57]. Most GBMs are diagnosed without an antecedent lower-grade tumor being detected – so-called *de novo* or primary GBMs, which are seen more commonly in the elderly. Primary (*de novo*) GBMs often display loss of PTEN (phosphatase and tensin homolog deleted on chromosome 10) and amplification, mutation or overexpression of the EGFR. Other molecular abnormalities in primary GBMs involve mutation or deletion of $p14^{ARF}$ and $p16^{INK4A}$, which are co-localized on chromosome 9p. A smaller fraction of high-grade astrocytomas, termed secondary GBMs, present initially at a lower-grade tumor, with subsequent progression to higher grades. Primary and secondary GBMs may be associated with different frequencies of molecular changes [9]. Low-grade, diffuse astrocytomas (WHO grade II) often display mutations of tumor-suppressor gene TP53 and overexpression of platelet-derived growth factor (PDGF) ligands and their cognate receptors [58]. Progression to anaplastic astrocytoma (WHO grade III) is associated with several cellular alterations including deletion or mutation of cyclin-dependent kinase inhibitor $p16^{INK4A}$ or the retinoblastoma susceptibility locus 1 (pRB1), as well as amplification or overexpression of cyclin-dependent kinase 4 and human double minute 2 (human xenolog of mouse double minute 2). Transformation to GBMs is associated with deletion of chromosome 10. Angiogenic cytokine profiles are also different between primary and secondary GBMs with predominant vascular endothelial growth factor (VEGF)-A expression in primary GBMs and high expression of PDGF-AB in secondary GBMs [59]. Recent large-scale expression analyses have defined the gene expression differences that distinguish primary and secondary glioblastomas [60]. Secondary glioblastoma-associated genes primarily include mitotic cell cycle components, suggesting the loss of function in prominent cell cycle regulators, whereas primary glioblastoma-associated genes demonstrate genes typical of a stromal response, suggesting the importance of extracellular signaling. These data highlight that the development of gene pathway-targeted therapies may need to be specifically tailored to each subtype of glioblastoma.

Molecularly targeted therapy

Malignant gliomas are strikingly heterogeneous tumors in terms of 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 most malignant gliomas, leading to the emerging trials of novel targeted therapeutic agents in clinic (Fig. 1 and Table 1). Development of targeted therapy for gliomas must incorporate several issues, including adequate agent delivery to the tumor, specificity to tumor cells or tumor-associated endothelial cells relative to normal cells, and limited toxicity. Several classes of agents, such as low-molecular-weight kinase inhibitor and monoclonal antibodies, have been developed for therapy in malignant gliomas.

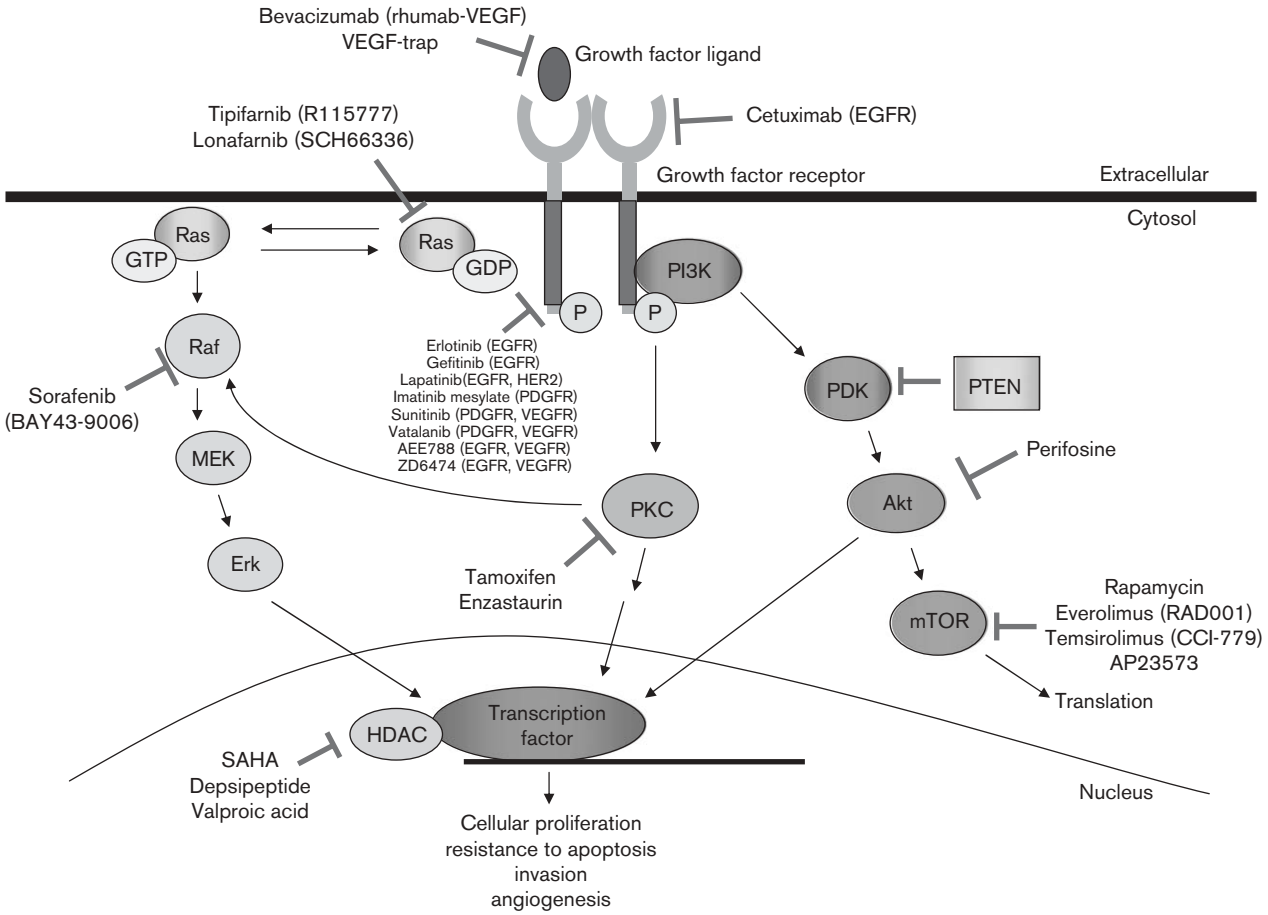
Kinase inhibitors

Most growth factor receptors initiate intracellular signaling mediated by associated tyrosine or serine/threonine kinase activities. The kinase regions catalyze transfer of phosphate groups from adenosine triphosphate (ATP) to transmembrane receptors or signal transducers to initiate signaling pathway activation. Selective kinase inhibitors were developed from screening large chemical libraries of ATP mimetics. The development of kinase inhibitors in cancer treatment has been stimulated by the remarkable success of imatinib mesylate in treating chronic myeloid leukemia and gastrointestinal stromal tumors. Imatinib mesylate specifically inhibits kinase activities associated with bcr-abl, c-kit and PDGF receptor (PDGFR) that are dysregulated in these cancers. Lessons derived from the efficacy of imatinib mesylate have generated new areas of research using kinase inhibitors to target specific aberrant pathways in many cancers, including malignant gliomas. Cancer cells may be exquisitely dependent on oncogenic pathways – 'oncogenic addiction' – prompting them to undergo apoptosis, whereas normal cells suffer only cell cycle arrest. Thus, specific disruption of aberrant molecular pathways may dramatically alter patient outcome with cancers dependent on these pathways.

Epidermal growth factor receptor

EGFR is amplified in approximately 50% of GBMs and is overexpressed in many malignant gliomas regardless of amplification status [61]. EGFR gene amplification is also associated with poor prognosis in patients with GBM [62]. In addition, frequent overexpression of several mutant forms of EGFR, including EGFRvIII (EGFRΔ2–7), suggests that EGFR is a key factor in tumorigenesis and provides a rationale for the use of EGFR-targeted therapies in these patients [63]. Two small-molecule inhibitors of EGFR, erlotinib (Tarceva, OSI-774; Genentech) and gefitinib (Iressa, ZD1839; AstraZeneca), have been widely tested in human malignancies, including malignant gliomas. In our recently published phase II trial of gefitinib for recurrent GBM patients, the median event-free survival was only 8.1 weeks and no radiographic responses were observed, although nine of 53 patients (17%) remained progression-free for at least 6 months [64]. Similarly, in a phase I trial of erlotinib, when used either alone or in combination with temozolomide, erlotinib demonstrated partial response in eight patients (14%) and 11% of patients remained progression-free at 6 months [31]. In an ongoing phase II study of erlotinib in patients with recurrent GBM, the overall response rate was 25%, with an additional 25% of patients having stable disease [65]. In another phase II study, however, the median progression-free survival of erlotinib-treated patients was only 12 weeks [66]. Clinical and radiographic

Fig. 1



Molecularly targeted therapies against glioma cells and/or tumor-associated endothelial cells. Malignant glioma cells and associated endothelial cells often have constitutive activation of the pathways of several growth factor receptors such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR). Each growth factor family consists of several members for which cognate receptors are transmembrane glycoproteins containing extracellular ligand-binding domains, hydrophobic membrane-spanning regions and cytoplasmic domains having protein-tyrosine kinase activity. Ligand binding to receptors induces receptor dimerization and phosphorylation (P). This receptor activation can induce the activity of numerous intracellular signal transduction pathways that regulate gene transcription of essential cellular proteins, including RAS, mitogen-activated protein kinase (MAPK; also known as extracellular signal-related protein Kinase; ERK) and Akt. Several points in these cascades are the targets of therapies in development for gliomas, some of which are shown. Most of these agents have proven to be well tolerated, but with limited activity against tumor as single agents. Multi-targeted kinase inhibitors, combinations of single kinase inhibitors, and combinations of these novel agents with radiation therapy and/or conventional chemotherapy may improve antitumor efficacy. HDAC, histone deacetylase; MEK, MAPK/ERK kinase; mTOR, mammalian target of rapamycin; PDK, 3-phosphoinositide-dependent protein kinase; PKC, protein kinase C; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SAHA, suberoylanilide hydroxamic acid; rhumab-VEGF, recombinant human monoclonal antibody to VEGF.

responses to EGFR kinase inhibitors in lung cancers have been linked to kinase region mutations in the EGFR molecule. We and others did not detect these mutations in our glioma specimens, but a comprehensive evaluation has not been performed to date. Two recent, elegant studies demonstrated that amplification or high expression of wild-type EGFR and low levels of phosphorylated Akt/protein kinase B in one study [67] and co-expression of EGFRvIII and wild-type phosphatase and tensin homolog (PTEN) in another study [68] were associated with increased 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, although it remains probable that these agents will modify disease outcome only when used in combination with other therapies.

Platelet-derived growth factor receptors

PDGFRs are commonly expressed in glioma specimens and models of gliomagenesis have been generated through modulation of PDGF signaling [58]. The PDGFR inhibitor, imatinib mesylate, exhibited antiglioma activity in preclinical studies [69]. In addition, imatinib mesylate also sensitized glioma cells to radiation injury [70]. Despite success in preclinical studies,

Table 1 Summary of molecularly targeted therapeutics in malignant gliomas

Agents	Targets	Stage of development	Response rate
Gefitinib	EGFR	phase II	recurrent GBM: 6-month, PFS 17%; no radiographic response
Gefitinib plus rapamycin	EGFR and mTOR	phase I	recurrent MG: MTDs identified; 6% PR; 38% SD
Erlotinib (±temozolomide)	EGFR	phase I and II	recurrent GBM: 6-month PFS 11%; 10–20% PR; 25% SD
Erlotinib plus temsirolimus	EGFR and mTOR	phase I/II	recurrent GBM: ongoing
Bevacizumab plus irinotecan	VEGF	phase II	recurrent MG: ongoing
Imatinib plus hydroxyurea	PDGFR	phase II	recurrent GBM: 6-month PFS: 27%; 9% PR; 42% SD
Vatalanib (PTK787)	PDGFR	phase I/II	recurrent GBM: ongoing
AEE788	EGFR,VEGFR	phase I	recurrent GBM: ongoing
AEE788 plus everolimus	EGFR, VEGFR, mTOR	phase I	recurrent GBM: ongoing
Tipifarnib	farnesyltransferase	phase I/II	recurrent MG: MTD reached; modest efficacy
Lonafarnib plus temozolomide	farnesyltransferase	phase I	recurrent MG: ongoing
AP23573	mTOR	phase I (with surgery)	target validation with in-vivo inhibition of mTOR; no radiographic response
Temsirolimus	mTOR	phase II	recurrent GBM: 6-month PFS 7.8%; 36% radiographic improvement
2-Methoxyestradiol	microtubule	phase II	recurrent GBM: ongoing
SAHA	HDAC	phase I/II	recurrent MG: ongoing
Depsipeptide	HDAC	phase I/II	recurrent MG: ongoing
Sorafenib	RAF, VEGFR	phase I/II	recurrent GBM: ongoing
Enzastaurin (LY317615)	PKC-β	phase II	recurrent MG: ongoing; 18% radiographic response
Cilengitide	integrins	phase I	recurrent GBM: completed accrual
Marimastat plus temozolomide	MMPs	phase II	recurrent GBM: 6-month PFS: 39%; 14% PR; 59% SD
AP12009	TGF-β	phase I	recurrent GBM: median survival 47 weeks
ZD6474	EGFR, VEGFR	preclinical	clinical trial under development
Sunitinib	PDGFR, VEGFR	preclinical	clinical trial under development
Perifosine	Akt	preclinical	clinical trial under development

EGFR, epidermal growth factor receptor; GBM, glioblastoma multiforme; PFS, progression-free survival; mTOR, mammalian target of rapamycin; MG, malignant glioma; MTD, maximal tolerated dose; PR, partial response; SD, stable disease; VEGF, vascular endothelial growth factor; PDGFR, platelet-derived growth factor receptor; VEGFR, VEGF receptor; SAHA; suberoylanilide hydroxamic acid; HDAC, histone deacetylase; PKC-β; protein kinase C-β; MMP, matrix metalloproteinase; TGF-β, transforming growth factor-β.

imatinib mesylate monotherapy failed to show clinical benefits in several phase II trials [71,72]. The combination of imatinib mesylate and hydroxyurea, however, has demonstrated more promising results in one patient series [73], which was confirmed by a subsequent phase II trial in recurrent GBM patients [74]. The 6-month progression-free survival of this combination was 27%, with the median progression-free survival of 14 weeks. Nine percent of patients had radiographic response, while 42% achieved stable disease at the median follow-up of 58 weeks [74]. The mechanism of antitumor activity of this combination remains unknown. Given the encouraging results of imatinib mesylate plus hydroxyurea, several combinations of imatinib mesylate with other chemotherapies such as temozolomide [30] are under clinical investigation.

Vascular endothelial growth factor receptor

One of the hallmarks of cancer is the ability to form new blood vessels (neoangiogenesis) [75]. VEGF is a heparin-binding growth factor that plays a pivotal role in neoangiogenesis [76]. Glioma tumor cells secrete VEGF, whereas tumor-associated endothelial cells express high levels of VEGFR receptor (VEGFR)-2 (also known as kinase insert domain receptor; KDR), creating a paracrine loop of angiogenesis activation. VEGF promotes endothelial cell proliferation, migration and tube formation. Pharmacological inhibition of VEGFR-2 by small molecule inhibitors blocks growth of malignant glioma in preclinical models [77,78]. On the basis of these encouraging results, several clinical trials of angiogenic kinase inhibitors have been initiated. Vatalanib (PTK787/ZK222584; Novartis), a kinase inhibitor of VEGFR and PDGFR, was evaluated in multi-centered phase I/II trials alone or in combination with chemotherapy. Vatalanib monotherapy in recurrent malignant gliomas demonstrated 4% partial response, 66% stable disease and 30% progressive disease [79]. A combination study of vatalanib with either temozolomide or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (lomustine) demonstrated that both combinations were well tolerated, but the efficacy appeared modest with time to progression of 16 weeks for temozolomide plus vatalanib and 12 weeks for CCNU plus vatalanib [80].

Multi-targeted kinase inhibitors

Failure of the first generation of targeted agents in many solid cancers may be due to the existence of multiple parallel or compensatory oncogenic pathways that allow tumor cells to escape and survive. Newer-generation small-molecule kinase inhibitors are designed to target multiple aberrant signaling pathways in the tumors and tumor-associated vasculature. Several small-molecule kinase inhibitors disrupting the functions of both EGFR and VEGFR potentially affecting both tumor cells and tumor-associated endothelial cells have been developed. AEE788 (Novartis) is a dual-kinase inhibitor of EGFR and VEGFR-2. AEE788 has efficacy in both primary glioma cell lines and a murine model of glioblastoma [81,82]. Multi-center trials of AEE788 alone or in combination with RAD001 [everolimus, a mammalian target of rapamycin (mTOR) inhibitor; Novartis] in recurrent malignant glioma are ongoing [83]. ZD6474 (Vandetanib, Zactima, AstraZeneca), a kinase inhibitor of VEGFR-2 and EGFR, displayed survival benefits in murine models of intracranial human glioma xenografts

[84] and a clinical trial of ZD6474 in malignant glioma is under development. The efficacy of AEE788 and ZD6474 in preclinical models was greater than an inhibitor of either EGFR or VEGFR in identical models, suggesting that simultaneous pathway disruption may offer additional benefit. Sunitinib (SU11248; Pfizer), a multitargeted kinase inhibitor of VEGFR-2, PDGFR, c-kit and fetal liver tyrosine kinase 3, was active against a subcutaneous model of glioblastoma [85]. A phase I study of sunitinib in advanced solid malignancies has been completed [86], and a phase II study in malignant glioma is under development. Sorafenib (Nexavar, BAY 43-9006; Bayer, West Haven, Connecticut, USA and Onyx, Emeryville, California, USA) is a kinase inhibitor of RAF, VEGFR and PDGFR with broad-spectrum antitumor activity in several cancer cell lines [87]. Although most GBMs lack RAF mutations, targeting the RAF/MEK/ERK pathway may be beneficial as this pathway may be activated by other genetic alterations. Sorafenib was well tolerated in a phase I study in advanced solid tumors [88]. The NABTC is conducting multiple phase I/II studies of sorafenib in combination with erlotinib (OSI-779, Tarceva; Genentech), the farnesyltransferase inhibitor tipifarnib (R115777, Zarnestra; Johnson & Johnson, New Brunswick, New Jersey, USA) or the mTOR inhibitor temsirolimus (CCI-779; Wyeth, Madison, New Jersey, USA). Lapatinib (GW572016; Glaxo-Smith-Kline, Research Triangle Park, North Carolina, USA) is an ERBB1/EGFR and ERBB2/HER-2 kinase inhibitor that demonstrated tolerability and encouraging clinical benefits in a recent phase I trial of metastatic cancers with either EGFR or ERBB-2 expression [89]. A phase II trial in recurrent malignant gliomas is planned.

Monoclonal antibodies

Monoclonal antibodies recognize antigenic epitopes with high selectivity and affinity and have shown promise in the treatment of several cancers. The main obstacle for the use of monoclonal antibodies in malignant gliomas may be limited delivery. Typically, monoclonal antibodies are large, adhesive molecules that do not penetrate an intact blood–brain barrier. Several strategies may be used in conjunction with monoclonal antibody administration, such as osmotic blood–brain barrier disruption, or local delivery into the tumor/resection cavity or CED, as discussed above. Targeting angiogenesis through systemic anti-VEGF therapies subverts delivery limitations, as a monoclonal antibody may only require intraluminal vascular delivery to exert antiangiogenic effect without crossing the blood–brain barrier.

Bevacizumab

Bevacizumab (Avastin; Genentech) is a recombinant human monoclonal antibody to VEGF. A murine monoclonal antibody to VEGF (A4.6.1) was developed in the early 1990s [90]. Its recombinant human counterpart, bevacizumab, has now become widely used in treatment for several advanced solid malignancies in combination with chemotherapies [91,92]. In a rat intracranial C6 glioblastoma model, A4.6.1 treatment decreased tumor vascularity, enhanced tumor apoptosis and prolonged survival [93]. The antitumor mechanism of bevacizumab is unclear. Decreased vessel diameter and reduction in interstitial fluid pressure were observed [94]. 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 [94]. Stark-Vance [95] recently reported dramatic efficacy of bevacizumab plus irinotecan for patients with recurrent GBM. In this case series of 29 heavily pretreated patients with recurrent GBM, 16 patients achieved radiographic response (55%), 10 achieved stable disease (34%) and only three developed progressive disease (10%). The regimen was well tolerated overall, although one intra-cerebral hemorrhage was reported. A phase II trial of this combination at Duke has been completed. The preliminary data confirms the efficacy of this combination (J. Vredenburgh, in preparation). Future studies of bevacizumab in combination with radiation therapy, other chemotherapies or small-molecule kinase inhibitors are being developed.

Miscellaneous

Anti-invasion agents

Several new therapeutic agents have been developed to modify the malignant phenotypes (such as invasion and apoptosis) of cancers. Tumor invasion of the normal brain is a hallmark of malignant gliomas that contributes to therapeutic resistance in multiple ways. Tumor cell migration is a complex, dynamic interaction of multiple cellular processes, including the alteration of tumor cell adhesion to a modified extracellular matrix, the secretion of proteases by the cells and modifications to the actin cytoskeleton (motility) [96]. High expression of genes upregulating the invasion correlates with poor clinical outcome [97]. Thus, targeting migration/invasion might offer benefits for patients with malignant gliomas. Cilengitide (EMD121974; EMD Pharmaceuticals, Durham, North Carolina, USA), an intravenous integrin inhibitor, showed preclinical activity in malignant gliomas [98] and phase II clinical trials of cilengitide are ongoing. Cilengitide may target both tumor cells and associated vasculature. Proteases such as MMPs secreted by tumor cells are critical enzymes that disrupt the extracellular matrix to facilitate the cellular migration. A phase II trial of an MMP inhibitor, marimastat, plus temozolomide in patients with recurrent malignant gliomas showed encouraging clinical activity with the 6-month progression-free survival of 39% [29]. Side-effects of severe joint and tendon pain in more than half of the patients tested, however, may limit the use of marimastat. Dasatinib (BMS-354825; Bristol-Myers Squibb, New York, New York, USA), a selective Src/abl kinase inhibitor, is under

clinical development for malignant gliomas. Other targets, such as plasminogen activator inhibitor-1 and secreted protein acidic and rich in cysteine (osteonectin), are under preclinical investigation.

Agents targeting apoptosis pathways

Apoptosis, commonly called programmed cell death in developmental biology, is a major mechanism of cell death in response to many toxic stimuli, including withdrawal of external survival signals and DNA damage. Resistance to apoptosis is one of the hallmarks of cancer. Several agents directly targeting apoptosis pathways have been tested in malignant gliomas with only modest efficacy. Other agents that indirectly target or modulate the apoptosis pathways include proteosome inhibitors, nuclear factor-κB inhibitors phosphatidylinositol-3 kinase (PI3K)/Akt inhibitors and mTOR inhibitors. These agents are in early clinical development. The ubiquitin–proteosome system is important in regulating the intracellular level of proteins, hence balancing cell proliferation and apoptosis [99]. Bortezomib (Velcade, PS-341; Millennium Pharmaceuticals, Cambridge, Massachusetts, USA), a proteosome inhibitor, induced growth arrest and apoptosis in glioma cell lines and explant [100], and is currently in a phase I/II study in malignant gliomas. The mTOR (also known as FK-506-binding protein 12-rapamycin-associated protein 1) is a downstream component of the PI3K/Akt pathway. mTOR regulates proliferation by activating downstream protein kinases required for ribosomal biosynthesis and mRNA translation, driving cell cycle progression. 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. Activation of the PI3K pathway by overexpression of upstream growth factor receptors (e.g. EGFR and/or deletion of PTEN) is significantly associated with increasing tumor grade, decreased levels of apoptosis and adverse clinical outcome in human gliomas [101]. Therefore, targeting 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), CCI-779 (temsirolimus; Wyeth), RAD001 (everolimus; Novartis), and AP23573 (Ariad Pharmaceuticals, Cambridge, Massachusetts, USA). A phase II study of temsirolimus in recurrent GBMs demonstrated modest efficacy with some evidence of activity based on molecular phenotype [102]. Several inhibitors of the PI3K/Akt pathway have been developed. Perifosine (KRX-0401; Keryx Biopharmaceuticals, New York, New York, USA), an orally available Akt/AMPK inhibitor, has shown promising preclinical activity when combined with temozolomide in a transgenic glioma model [103]. Perifosine is now under clinical trial development in malignant glioma. In addition, new agents modulating apoptosis pathways such as heat shock protein 70 (Hsp70) inducers and poly(ADP-ribose) polymerase (PARP) inhibitor are undergoing preclinical or early clinical development in malignant gliomas.

Farnesyltransferase inhibitors

Signal transduction from activated receptor tyrosine kinases is partly mediated through the RAS/RAF/MEK/ERK pathway. Malignant gliomas exhibit activity of three major types of RAS: N-RAS, H-RAS and K-RAS [104]. Active RAS is dependent on posttranslational modifications to correctly target the protein to membranes where signaling complexes are generated. The rate-limiting step involves the addition of prenyl groups – either farnesyl or geranylgeranyl groups. Many other proteins undergo parallel modifications. Farnesyltransferase inhibitors (FTIs) have been developed that may function by blocking RAS activity or the activities of other proteins yet to be fully defined. FTIs have activity against human gliomas and two specific FTIs (tipifarnib, Zarnestra, R115777; Johnson & Johnson and lonafarnib, Sarasar, SCH66336; Schering-Plough) have been developed. The NABTC completed a phase I/II study of tipifarnib in recurrent malignant gliomas with a 6-month progression-free survival rate of 24% and only three partial response patients [105]. These results suggest modest efficacy of FTIs as monotherapy in recurrent malignant gliomas. Studies combining FTIs with radiation therapy, temozolomide and other targeted therapies are under development.

Protein kinase C inhibitors

The true molecular target of tamoxifen remains controversial. In addition to its estrogen agonist–antagonist functions, tamoxifen inhibits protein kinase C, insulin-like growth factor-II and nuclear factor-κB pathways, and it has activity against glioma xenograft *in vivo* [106]. Several studies have shown modest efficacy of tamoxifen alone [107] or in combination with chemotherapies, such as procarbazine [108] or temozolomide [109], in recurrent malignant gliomas. Activation of protein kinase Cβ has been implicated in neoangiogenesis, tumor cell proliferation, apoptosis and invasion. The selective protein kinase Cβ inhibitor, enzastaurin (LY317615; Eli Lilly, Indianapolis, Indiana, USA), demonstrated preclinical activity against glioblastoma xenograft [110] and is being tested in clinical trial for recurrent malignant gliomas with promising activity [111]. A multi-centered phase II trial is planned.

Deacetylase inhibitors

The histone code refers to specific modifications of nucleosome-associated histone proteins involved in the regulation of gene transcription. These modifications include the 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 acetyl transferases) or induce histone deacetylation (histone deacetylases; HDACs). The HDAC family includes cytoplastmic deacetylases (HDACs 4, 6 and 10) that function to regulate key cytoplasmic targets to regulate growth factor degradation [112], suggesting that non-histone targets may also contribute to the efficacy of deacetylase inhibitors. Inhibition of HDAC has been associated with cell differentiation and induction of apoptosis in malignant gliomas. Pretreatment with a HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA; Aton Pharma, Tarrytown, New York, USA), can sensitize glioma cells to chemotherapy [113] and radiation [114]. This agent and other deacetylase inhibitors, such as depsipeptide (FK228; Gloucester Pharmaceuticals, Cambridge, Massachusetts, USA), LBH589 (Novartis) and valproic acid, are under clinical development in malignant gliomas.

Heat shock protein 90 inhibitors

Hsp90 is an ATP-dependent molecular chaperone that stabilizes regulatory proteins [115]. Many HSP90 client proteins are involved in cell cycle regulation and cell survival, and are associated with a cytoprotection against DNA damage. Therefore, targeting this chaperone protein might act as a direct antitumor agent or enhance cytotoxicity of radiation and chemotherapy to cancer cells. A preclinical study revealed the ability of geldanamycin (Hsp90 protein disrupting agent) to inhibit the growth of glioma cells [116]. In addition, 17-allylamino-17-demethoxygeldanamycin, a less toxic and less potent derivative of geldanamycin, significantly inhibited the growth of a glioma xenograft in nude mice [116]. Recent data also showed enhanced tumor cell radiosensitivity by another orally available Hsp90 inhibitor, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin [117]. Clinical development of Hsp90 inhibitors in malignant gliomas appears warranted.

Angiogenesis inhibitors

In addition to monoclonal antibodies to VEGF and kinase inhibitors of VEGFR-2, several angiogenesis inhibitors of uncertain mechanism of action have been used in malignant gliomas. Thalidomide (Thalomid; Celgene, Summit, New Jersey, USA) has been tested in clinical trials for both monotherapy and in combination with chemotherapy. Thalidomide alone has modest activity against recurrent malignant gliomas [118]. Thalidomide in combination with chemotherapy such as carmustine [119] or temozolomide [120] appears to be more effective than either agent alone. Further clinical studies are needed to confirm the efficacy. Cyclooxygenase-2 inhibitors such as celecoxib have been tested in clinical trials of malignant gliomas with only modest efficacy [121]. Recent concerns over cardiovascular side-effects have limited the clinical use of these agents. Newer agents such as pan-VEGFR kinase inhibitors, soluble decoy receptors of VEGF (VEGF-trap) and angiopoietin inhibitors are currently under preclinical development.

Transforming growth factor-β inhibitors

Transforming growth factor (TGF)-β is a multifunctional cytokine expressed in malignant gliomas that increase tumor cell motility and invasion, angiogenesis and immune escape. Malignant glioma cells secrete TGF-β ligands and express receptors, indicating the presence of an autocrine loop. Preclinical studies using antisense oligonucleotides directed to mRNAs encoding TGF-β ligands have shown promise. AP12009 (Antisense Pharma, Regensburg, Germany), a TGF-β ligand-specific antisense oligonucleotide, was well tolerated when administered intratumorally. The median survival for GBM was 47 weeks [122]. Several small molecule ATP mimetics of TGF-β receptors have shown efficacy in preclinical studies of malignant gliomas [123,124]. These agents might have utility either as monotherapies, or in combination with chemotherapy or immunomodulatory treatment.

Other potential therapeutic agents

Several agents inhibiting novel targets are in preclinical development for the treatment of cancers including malignant gliomas. Cell cycle regulators such as cyclin-dependent kinase inhibitors, aurora kinase and polo-like kinase inhibitors, and mitotic kinesin inhibitors are under testing for several hematologic and solid malignancies. In addition, inhibitors disrupting both cell cycle regulators and receptor or angiogenic kinases have been developed.

Combination therapy and multi-modality treatment

Several potential resistance mechanisms to molecularly targeted therapeutic agents have been elucidated. Cell lines treated with growth factor receptor inhibitors usually regrow with increased expression of other mitogenic growth factors. Preclinical studies of glioma cell lines, which are resistant to EGFR kinase inhibitors, exhibited activation of the IGF1/PI3K/Akt pathway [125,126]. In addition, malignant gliomas are genetically heterogeneous even in single tumors; thus, targeting only one oncogenic pathway may not be sufficient to control tumor growth. Disrupting several growth factor pathways by multi-targeted kinase inhibitors or by the combination of several single-kinase inhibitors to block multiple mitogenic pathways might offer greater efficacy. We found 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 more than either agent alone [82]. An in-vivo preclinical study confirmed greater tumor

growth inhibition and greater increases in median survival than monotherapy [82]. Another study has also demonstrated the combinatorial benefit of this similar approach in a glioblastoma model by using an EGFR kinase inhibitor, EKI-785, and an mTOR inhibitor, rapamycin [127]. Several clinical studies based on this rationale of combination by targeting both upstream growth factor receptor signaling pathways and downstream PI3K pathway are ongoing. We recently completed a phase I trial of gefitinib plus rapamycin. The maximal tolerated dose of both agents was defined and encouraging antitumor activity was seen in some patients [128]. Phase I/II studies of erlotinib plus temsirolimus and AEE788 plus RAD001 are ongoing. Sequence of each drug administration is important to avoid negative interactions, e.g. some agents might require cells to be cycling to induce apoptosis, whereas others might only induce cell cycle arrest. On the basis of encouraging preliminary results of bevacizumab and irinotecan, combinations of such anti-angiogenic agents with chemotherapies or molecularly targeted therapeutics should be developed. Multi-modality treatment with conventional radiation or SRS should also be explored. Several lines of preclinical evidence show that small-molecule kinase inhibitors can sensitize tumor or have synergistic antitumor activity with radiation therapy [129–131]. Sequencing and timing of drug administration in relation to radiation treatment is crucial when designing multi-modality clinical trials [130,132]. Lastly, recent isolation of cancer stem cells from patients with GBM has created a paradigm shift in neuro-oncology research [133]. Cancer stem cells have been defined functionally and by stem cell marker expression. Cancer stem cells display self-renewal and multi-lineage differentiation potentials with heterogeneous tumor formation following transplantation of limited number of cells in immunocompromised rodents. These cancer stem cells form the tumors recapitulating parental histopathology when xenotransplanted into the mouse brain. As the molecular abnormalities of glioma stem cells are examined, targeted therapies aiming at these cells will be simultaneously developed. Targeting both stem-like and non-stem-like cancer cells may offer a new horizon in the treatment of these devastating tumors. These subsets of cancer cells are in contrast to engineered neural stem cells and bone marrow-derived stem cells that may track glioma cells to provide delivery vehicles for gene therapy or other agents [134,135].

Conclusion

The field of neuro-oncology has witnessed an explosive increase in new therapies undergoing laboratory and clinical evaluation. Although some therapies have been developed specifically for malignant gliomas, most therapies have been developed primarily for other solid cancers with greater patient numbers. The molecular heterogeneity of brain cancers makes an imatinib mesylate-like success unlikely, suggesting that combinations of targeted therapies with one another or cytotoxics will be required. The limited number of patients available for clinical trial in the face of many potential approaches will demand greater cooperation between centers and more rational trial designs, including tissue acquisition and pharmacokinetic studies. Eventual patient subgroup analysis may be supported, but the lack of definitive markers to date will require less selected populations until preliminary markers can be validated. Although brain cancers remain highly lethal, improved understanding of the underlying tumor biology and more selective antitumor therapies may offer patients new hope on the horizon. Continued efforts by those in neuro-oncology are required to help overcome repeated failures.

References

1 CBTRUS. *Statistical report: primary brain tumors in the United States, 1997–2001*. Chicago: Central Brain Tumor Registry of the United States (CBTRUS); 2005.

2 Percy C, Van Holten C, Muir CM, editors. *International classification of diseases for oncology*. 2nd ed. Geneva: World Health Organization; 1996.

3 Kleihues P, Burger PC, Scheithauer BW. The new WHO classification of brain tumours. *Brain Pathol* 1993; **3**:255–268.

4 McCormack BM, Miller DC, Budzilovich GN, Voorhees GJ, Ransohoff J. Treatment and survival of low-grade astrocytoma in adults: 1977–1988. *Neurosurgery* 1992; **31**:636–642.

5 Burger PC, Vogel FS, Green SB, Strike TA. Glioblastoma multiforme and anaplastic astrocytoma. Pathologic criteria and prognostic implications. *Cancer* 1985; **56**:1106–1111.

6 Scott CB, Scarantino C, Urtasun R, Movsas B, Jones CU, Simpson JR, *et al.* Validation and predictive power of Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis classes for malignant glioma patients: a report using RTOG 90-06. *Int J Radiat Oncol Biol Phys* 1998; **40**:51–55.

7 Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, *et al.* Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst* 1998; **90**:1473–1479.

8 Wong ET, Hess KR, Gleason MJ, Jaeckle KA, Kyritsis AP, Prados MD, *et al.* Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol* 1999; **17**:2572–2578.

9 Behin A, Hoang-Xuan K, Carpentier AF, Delattre JY. Primary brain tumours in adults. *Lancet* 2003; **361**:323–331.

10 Lacroix M, Abi-Said D, Fourney DR, Gokaslan ZL, Shi W, DeMonte F, *et al.* A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J Neurosurg* 2001; **95**: 190–198.

11 Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; **352**:987–996.

12 Taphoorn MJ, Stupp R, Coens C, Osoba D, Kortmann R, van den Bent MJ, *et al.* Health-related quality of life in patients with glioblastoma: a randomised controlled trial. *Lancet Oncol* 2005; **6**:937–944.

13 Yung WK, Prados MD, Yaya-Tur R, Rosenfeld SS, Brada M, Friedman HS, *et al.* Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group. *J Clin Oncol* 1999; **17**:2762–2771.

14 Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, *et al.* A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br J Cancer* 2000; **83**:588–593.

15 Friedman HS, McLendon RE, Kerby T, Dugan M, Bigner SH, Henry AJ, *et al.* DNA mismatch repair and O^6-alkylguanine-DNA alkyltransferase analysis and response to Temodal in newly diagnosed malignant glioma. *J Clin Oncol* 1998; **16**:3851–3857.

16 Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; **352**:997–1003.

17 Houghton PJ, Stewart CF, Cheshire PJ, Richmond LB, Kirstein MN, Poquette CA, *et al.* Antitumor activity of temozolomide combined with irinotecan is partly independent of O^6-methylguanine-DNA

methyltransferase and mismatch repair phenotypes in xenograft models. *Clin Cancer Res* 2000; **6**:4110–4118.
18 Friedman HS, Keir S, Pegg AE, Houghton PJ, Colvin OM, Moschel RC, *et al.* O^6-benzylguanine-mediated enhancement of chemotherapy. *Mol Cancer Ther* 2002; **1**:943–948.
19 Friedman HS, Kokkinakis DM, Pluda J, Friedman AH, Cokgor I, Haglund MM, *et al.* Phase I trial of O^6-benzylguanine for patients undergoing surgery for malignant glioma. *J Clin Oncol* 1998; **16**:3570–3575.
20 Friedman HS, Pluda J, Quinn JA, Ewesuedo RB, Long L, Friedman AH, *et al.* Phase I trial of carmustine plus O^6-benzylguanine for patients with recurrent or progressive malignant glioma. *J Clin Oncol* 2000; **18**: 3522–3528.
21 Quinn JA, Desjardins A, Weingart J, Brem H, Dolan ME, Delaney SM, *et al.* Phase I trial of temozolomide plus O^6-benzylguanine for patients with recurrent or progressive malignant glioma. *J Clin Oncol* 2005; **23**: 7178–7187.
22 Quinn JA, Pluda J, Dolan ME, Delaney S, Kaplan R, Rich JN, *et al.* Phase II trial of carmustine plus O^6-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma. *J Clin Oncol* 2002; **20**:2277–2283.
23 Newlands ES, Foster T, Zaknoen S. Phase I study of temozolomide (TMZ) combined with procarbazine (PCB) in patients with gliomas. *Br J Cancer* 2003; **89**:248–251.
24 Prados MD, Yung WK, Fine HA, Greenberg HS, Junck L, Chang SM, *et al.* Phase II study of BCNU and temozolomide for recurrent glioblastoma multiforme: North American Brain Tumor Consortium Study. *Neuro-Oncology* 2004; **6**:33–37.
25 Reardon DA, Quinn JA, Rich JN, Desjardins A, Vredenburgh J, Gururangan S, *et al.* Phase I trial of irinotecan plus temozolomide in adults with recurrent malignant glioma. *Cancer* 2005; **104**:1478–1486.
26 Yung WKA, Lieberman FS, Wen P, Robin I, Gilbert M, Chang S, *et al.* Combination of temozolomide (TMZ) and irinotecan (CPT-11) showed enhanced activity for recurrent malignant gliomas: a North American Brain Tumor Consortium (NABTC) phase II study. *Proc Am Soc Clin Oncol* 2005; **23** (**Suppl 119S**):1521.
27 Korones DN, Benita-Weiss M, Coyle TE, Mechtler L, Bushunow P, Evans B, *et al.* Phase I study of temozolomide and escalating doses of oral etoposide for adults with recurrent malignant glioma. *Cancer* 2003; **97**:1963–1968.
28 Jaeckle KA, Hess KR, Yung WK, Greenberg H, Fine H, Schiff D, *et al.* Phase II evaluation of temozolomide and 13-*cis*-retinoic acid for the treatment of recurrent and progressive malignant glioma: a North American Brain Tumor Consortium study. *J Clin Oncol* 2003; **21**:2305–2311.
29 Groves MD, Puduvalli VK, Hess KR, Jaeckle KA, Peterson P, Yung WK, *et al.* Phase II trial of temozolomide plus the matrix metalloproteinase inhibitor, marimastat, in recurrent and progressive glioblastoma multiforme. *J Clin Oncol* 2002; **20**:1383–1388.
30 Sathornsumetee S, Reardon DA, Quinn J, Rich JN, Vredenburgh JJ, Desjardins A, *et al.* A phase 1 dose escalation study of imatinib mesylate plus standard-dosed temozolomide in the treatment of patients with malignant glioma. *Proc Am Soc Clin Oncol* 2005; **23** (**Suppl 124S**):1540.
31 Prados MD, Lamborn KR, Chang S, Burton E, Butowski N, Malec M, *et al.* Phase 1 study of erlotinib HCl alone and combined with temozolomide in patients with stable or recurrent malignant glioma. *Neuro-Oncology* 2006; **8**:67–78.
32 Prados M, Yung W, Wen P, Junck L, Fink K, Cloughesy T, *et al.* Phase I study of ZD1839 plus temozolomide in patients with malignant glioma. a study of the North American Brain Tumor Consortium. *Proc Am Soc Clin Oncol* 2004; **22** (**Suppl 108S**):1504.
33 Friedman HS, Keir ST, Houghton PJ. The emerging role of irinotecan (CPT-11) in the treatment of malignant glioma in brain tumors. *Cancer* 2003; **97** (**Suppl**):2359–2362.
34 Houghton PJ, Cheshire PJ, Hallman JD 2nd, Lutz L, Friedman HS, Danks MK, *et al.* Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. *Cancer Chemother Pharmacol* 1995; **36**:393–403.
35 Friedman HS, Petros WP, Friedman AH, Schaaf LJ, Kerby T, Lawyer J, *et al.* Irinotecan therapy in adults with recurrent or progressive malignant glioma. *J Clin Oncol* 1999; **17**:1516–1525.
36 Batchelor TT, Gilbert MR, Supko JG, Carson KA, Nabors LB, Grossman SA, *et al.* Phase 2 study of weekly irinotecan in adults with recurrent malignant glioma: final report of NABTT 97-11. *Neuro-Oncology* 2004; **6**:21–27.
37 Madajewicz S, Chowhan N, Tfayli A, Roque C, Meek A, Davis R, *et al.* Therapy for patients with high grade astrocytoma using intraarterial chemotherapy and radiation therapy. *Cancer* 2000; **88**:2350–2356.
38 Wallner KE, Galicich JH, Krol G, Arbit E, Malkin MG. Pattern of failure following treatment for glioblastoma multiforme and anaplastic astrocytoma. *Int J Radiat Oncol Biol Phys* 1989; **16**:1405–1409.
39 Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, *et al.* A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-Oncology* 2003; **5**:79–88.
40 Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, *et al.* Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent malignant gliomas. The Polymer-Brain Tumor Treatment Group. *Lancet* 1995; **345**:1008–1012.
41 Colapinto EV, Lee YS, Humphrey PA, Zalutsky MR, Friedman HS, Bullard DE, *et al.* The localisation of radiolabelled murine monoclonal antibody 81C6 and its Fab fragment in human glioma xenografts in athymic mice. *Br J Neurosurg* 1988; **2**:179–191.
42 Lee YS, Bullard DE, Zalutsky MR, Coleman RE, Wikstrand CJ, Friedman HS, *et al.* Therapeutic efficacy of antiglioma mesenchymal extracellular matrix 131I-radiolabeled murine monoclonal antibody in a human glioma xenograft model. *Cancer Res* 1988; **48**:559–566.
43 Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE 2nd, *et al.* Phase II trial of murine ^{131}I-labeled antitenascin monoclonal antibody 81C6 administered into surgically created resection cavities of patients with newly diagnosed malignant gliomas. *J Clin Oncol* 2002; **20**:1389–1397.
44 Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE 2nd, *et al.* Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J Clin Oncol* 2006; **24**:115–122.
45 Chan TA, Weingart JD, Parisi M, Hughes MA, Olivi A, Borzillary S, *et al.* Treatment of recurrent glioblastoma multiforme with GliaSite brachytherapy. *Int J Radiat Oncol Biol Phys* 2005; **62**:1133–1139.
46 Gabayan AJ, Green SB, Sanan A, Jenrette J, Schultz C, Papagikos M, *et al.* GliaSite brachytherapy for treatment of recurrent malignant gliomas: a retrospective multi-institutional analysis. *Neurosurgery* 2006; **58**:701–709.
47 Debinski W, Gibo DM, Obiri NI, Kealiher A, Puri RK. Novel anti-brain tumor cytotoxins specific for cancer cells. *Nat Biotechnol* 1998; **16**:449–453.
48 Prados M, Kunwar S, Lang FF, Ram Z, Westphal M, Barnett G, *et al.* Final results of phase I/II studies of IL13-PE38QQR administered intratumorally (IT) and/or peritumorally (PT) via convection-enhanced delivery (CED) in patients undergoing tumor resection for recurrent malignant glioma. *Proc Am Soc Clin Oncol* 2005; **23** (**Suppl 115S**):1506.
49 Weber F, Asher A, Bucholz R, Berger M, Prados M, Chang S, *et al.* Safety, tolerability, and tumor response of IL4-Pseudomonas exotoxin (NBI-3001) in patients with recurrent malignant glioma. *J Neurooncol* 2003; **64**: 125–137.
50 Sampson JH, Akabani G, Archer GE, Bigner DD, Berger MS, Friedman AH, *et al.* Progress report of a Phase I study of the intracerebral microinfusion of a recombinant chimeric protein composed of transforming growth factor (TGF)-alpha and a mutated form of the Pseudomonas exotoxin termed PE-38 (TP-38) for the treatment of malignant brain tumors. *J Neurooncol* 2003; **65**:27–35.
51 Laske DW, Youle RJ, Oldfield EH. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat Med* 1997; **3**:1362–1368.
52 Cohen KA, Liu T, Bissonette R, Puri RK, Frankel AE. DAB389EGF fusion protein therapy of refractory glioblastoma multiforme. *Curr Pharm Biotechnol* 2003; **4**:39–49.
53 Tsao MN, Mehta MP, Whelan TJ, Morris DE, Hayman JA, Flickinger JC, *et al.* The American Society for Therapeutic Radiology and Oncology (ASTRO) evidence-based review of the role of radiosurgery for malignant glioma. *Int J Radiat Oncol Biol Phys* 2005; **63**:47–55.
54 Mahajan A, McCutcheon IE, Suki D, Chang EL, Hassenbusch SJ, Weinberg JS, *et al.* Case-control study of stereotactic radiosurgery for recurrent glioblastoma multiforme. *J Neurosurg* 2005; **103**:210–217.
55 Combs SE, Widmer V, Thilmann C, Hof H, Debus J, Schulz-Ertner D. Stereotactic radiosurgery (SRS): treatment option for recurrent glioblastoma multiforme (GBM). *Cancer* 2005; **104**:2168–2173.
56 Combs SE, Thilmann C, Edler L, Debus J, Schulz-Ertner D. Efficacy of fractionated stereotactic reirradiation in recurrent gliomas: long-term results in 172 patients treated in a single institution. *J Clin Oncol* 2005; **23**:8863–8869.
57 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**:57–70.
58 Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces

oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes *in vivo*. *Genes Dev* 2001; **15**:1913–1925.
59 Karcher S, Steiner HH, Ahmadi R, Zoubaa S, Vasvari G, Bauer H, *et al.* Different angiogenic phenotypes in primary and secondary glioblastomas. *Int J Cancer* 2005; **118**:2182–2189.
60 Tso CL, Freije WA, Day A, Chen Z, Merriman B, Perlina A, *et al.* Distinct transcription profiles of primary and secondary glioblastoma subgroups. *Cancer Res* 2006; **66**:159–167.
61 Bigner SH, Humphrey PA, Wong AJ, Vogelstein B, Mark J, Friedman HS, *et al.* Characterization of the epidermal growth factor receptor in human glioma cell lines and xenografts. *Cancer Res* 1990; **50**:8017–8022.
62 Smith JS, Tachibana I, Passe SM, Huntley BK, Borell TJ, Iturria N, *et al.* PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. *J Natl Cancer Inst* 2001; **93**:1246–1256.
63 Kuan CT, Wikstrand CJ, Bigner DD. EGF mutant receptor vIII as a molecular target in cancer therapy. *Endocr Relat Cancer* 2001; **8**:83–96.
64 Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, *et al.* Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004; **22**: 133–142.
65 Vogelbaum MA, Peereboom D, Stevens G, Barnett G, Brewer C. Phase II trial of EGFR tyrosine kinase inhibitor erlotinib for single agent therapy of recurrent glioblastoma multiforme: interim results. *Proc Am Soc Clin Oncol* 2004; **22**:1558a.
66 Raizer JJ, Abrey LE, Wen P, Cloughesy T, Robins IA, Fine HA, *et al.* Phase II trial of erlotinib (OSI-779) in patients (pts) with recurrent malignant gliomas (MG) not on EIAEDs. *Proc Am Soc Clin Oncol* 2004; **22**:1502a.
67 Haas-Kogan DA, Prados MD, Tihan T, Eberhard DA, Jelluma N, Arvold ND, *et al.* Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. *J Natl Cancer Inst* 2005; **97**:880–887.
68 Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, *et al.* Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 2005; **353**:2012–2024.
69 Kilic T, Alberta JA, Zdunek PR, Acar M, Iannarelli P, O'Reilly T, *et al.* Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class. *Cancer Res* 2000; **60**:5143–5150.
70 Russell JS, Brady K, Burgan WE, Cerra MA, Oswald KA, Camphausen K, *et al.* Gleevec-mediated inhibition of Rad51 expression and enhancement of tumor cell radiosensitivity. *Cancer Res* 2003; **63**:7377–7383.
71 Wen PY, Yung WKA, Lamborn K, Peng B, Dahia P, Abrey L, *et al.* Phase I/II study of imatinib mesylate (STI571) for patients with recurrent gliomas (NABTC 99-08). *Neuro-Oncology* 2004; **6**:385.
72 Van den Bent M, Brandes AA, van Oosterom A, Dittrich C, Fumoleau P, Coudert B, *et al.* Multicentre phase II study of imatinib mesylate (Gleevec) in patients with recurrent glioblastoma: an EORTC NDDG/BTG intergroup study. *Neuro-Oncology* 2004; **6**:383–384.
73 Dresemann G. Imatinib and hydroxyurea in pretreated progressive glioblastoma multiforme: a patient series. *Ann Oncol* 2005; **16**:1702–1708.
74 Reardon DA, Egorin MJ, Quinn JA, Rich JN, Gururangan S, Vredenburgh JJ, *et al.* Phase 2 study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. *J Clin Oncol* 2005; **23**:9359–9368.
75 Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**:1182–1186.
76 Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; **246**:1306–1309.
77 Laird AD, Vajkoczy P, Shawver LK, Thurnher A, Liang C, Mohammadi M, *et al.* SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res* 2000; **60**:4152–4160.
78 Goldbrunner RH, Bendszus M, Wood J, Kiderlen M, Sasaki M, Tonn JC. PTK787/ZK222584, an inhibitor of vascular endothelial growth factor receptor tyrosine kinases, decreases glioma growth and vascularization. *Neurosurgery* 2004; **55**:426–432.
79 Conrad C, Friedman H, Reardon D, Provenzale J, Jackson E, Serajuddin H, *et al.* A phase I/II trial of single-agent PTK 787/ZK 222584 (PTK/ZK), a novel, oral angiogenesis inhibitor, in patients with recurrent glioblastoma multiforme (GBM). *Proc Am Soc Clin Oncol* 2004; **22** (**Suppl 110S**):1512.
80 Reardon D, Friedman H, Yung WKA, Brada M, Conrad C, Provenzale J, *et al.* A phase I/II trial of PTK787/ZK 222584 (PTK/ZK), a novel, oral angiogenesis inhibitor, in combination with either temozolomide or lomustine for patients with recurrent glioblastoma multiforme (GBM). *Proc Am Soc Clin Oncol* 2004; **22** (**Suppl 110S**):1513.
81 Traxler P, Allegrini PR, Brandt R, Brueggen J, Cozens R, Fabbro D, *et al.* AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res* 2004; **64**:4931–4941.
82 Goudar RK, Shi Q, Hjelmeland MD, Keir ST, McLendon RE, Wikstrand CJ, *et al.* Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. *Mol Cancer Ther* 2005; **4**:101–112.
83 Reardon D, Cloughesy T, Conrad C, Prados M, Xia J, Mietlowski W, *et al.* A phase I study of AEE788, a novel multitargeted inhibitor of ErbB and VEGF receptor family tyrosine kinases, in recurrent GBM patients. *Proc Am Soc Clin Oncol* 2005; **23** (**Suppl 207S**):3036.
84 Rich JN, Sathornsumetee S, Keir ST, Kieran MW, Laforme A, Kaipainen A, *et al.* ZD6474, a novel tyrosine kinase inhibitor of vascular endothelial growth factor receptor and epidermal growth factor receptor, inhibits tumor growth of multiple nervous system tumors. *Clin Cancer Res* 2005; **11**:8145–8157.
85 Schueneman AJ, Himmelfarb E, Geng L, Tan J, Donnelly E, Mendel D, *et al.* SU11248 maintenance therapy prevents tumor regrowth after fractionated irradiation of murine tumor models. *Cancer Res* 2003; **63**:4009–4016.
86 Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, *et al.* Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006; **24**:25–35.
87 Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, *et al.* BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**:7099–7109.
88 Strumberg D, Richly H, Hilger RA, Schleucher N, Korfee S, Tewes M, *et al.* Phase I clinical and pharmacokinetic study of the novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. *J Clin Oncol* 2005; **23**:965–972.
89 Burris HA 3rd, Hurwitz HI, Dees EC, Dowlati A, Blackwell KL, O'Neil B, *et al.* Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J Clin Oncol* 2005; **23**:5305–5313.
90 Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, *et al.* Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature* 1993; **362**:841–844.
91 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, *et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**:2335–2342.
92 Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, *et al.* Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004; **22**:2184–2191.
93 Rubenstein JL, Kim J, Ozawa T, Zhang M, Westphal M, Deen DF, *et al.* Anti VEGF-antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. *Neoplasia* 2000; **2**:306–314.
94 Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. *Cancer Res* 2005; **65**:671–680.
95 Stark-Vance V. Bevacizumab and CPT-11 in the treatment of relapsed malignant glioma. *Neuro-Oncology* 2005; **7**:369.
96 Lefranc F, Brotchi J, Kiss R. Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. *J Clin Oncol* 2005; **23**: 2411–2422.
97 Rich JN, Hans C, Jones B, Iversen ES, McLendon RE, Rasheed BK, *et al.* Gene expression profiling and genetic markers in glioblastoma survival. *Cancer Res* 2005; **65**:4051–4058.
98 Taga T, Suzuki A, Gonzalez-Gomez I, Gilles FH, Stins M, Shimada H, *et al.* Alpha v-integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. *Int J Cancer* 2002; **98**:690–697.
99 Mani A, Gelmann EP. The ubiquitin–proteasome pathway and its role in cancer. *J Clin Oncol* 2005; **23**:4776–4789.
100 Yin D, Zhou H, Kumagai T, Liu G, Ong JM, Black KL, *et al.* Proteasome inhibitor PS-341 causes cell growth arrest and apoptosis in human glioblastoma multiforme (GBM). *Oncogene* 2005; **24**:344–354.
101 Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A, *et al.* The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. *J Clin Oncol* 2004; **22**:1926–1933.

102 Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, *et al.* Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group study. *J Clin Oncol* 2005; **23**:5294–5304.

103 Momota H, Nerio E, Holland EC. Perifosine inhibits multiple signaling pathways in glial progenitors and cooperates with temozolomide to arrest cell proliferation in gliomas *in vivo*. *Cancer Res* 2005; **65**:7429–7435.

104 Guha A, Feldkamp MM, Lau N, Boss G, Pawson A. Proliferation of human malignant astrocytomas is dependent on Ras activation. *Oncogene* 1997; **15**:2755–2765.

105 Cloughesy TF, Kuhn J, Robins HI, Abrey L, Wen P, Fink K, *et al.* Phase I trial of tipifarnib in patients with recurrent malignant glioma taking enzyme-inducing antiepileptic drugs: a North American Brain Tumor Consortium study. *J Clin Oncol* 2005; **23**:6647–6656.

106 Hui AM, Zhang W, Chen W, Xi D, Purow B, Friedman GC, *et al.* Agents with selective estrogen receptor (ER) modulator activity induce apoptosis *in vitro* and *in vivo* in ER-negative glioma cells. *Cancer Res* 2004; **64**:9115–9123.

107 Chamberlain MC, Kormanik PA. Salvage chemotherapy with tamoxifen for recurrent anaplastic astrocytomas. *Arch Neurol* 1999; **56**:703–708.

108 Brandes AA, Ermani M, Turazzi S, Scelzi E, Berti F, Amista P, *et al.* Procarbazine and high-dose tamoxifen as a second-line regimen in recurrent high-grade gliomas: a phase II study. *J Clin Oncol* 1999; **17**:645–650.

109 Spence AM, Peterson RA, Scharnhorst JD, Silbergeld DL, Rostomily RC. Phase II study of concurrent continuous temozolomide (TMZ) and tamoxifen (TMX) for recurrent malignant astrocytic gliomas. *J Neurooncol* 2004; **70**:91–95.

110 Graff JR, McNulty AM, Hanna KR, Konicek BW, Lynch RL, Bailey SN, *et al.* The protein kinase Cbeta-selective inhibitor, enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. *Cancer Res* 2005; **65**:7462–7469.

111 Fine HA, Kim L, Royce C, Draper D, Haggarty I, Ellinzano H, *et al.* Results from phase II trial of enzastaurin (LY317615) in patients with recurrent high grade gliomas. *Proc Am Soc Clin Oncol* 2005; **23** (**Suppl 115S**):1504.

112 Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell* 2003; **115**:727–738.

113 Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Cancer Res* 2003; **63**:7291–7300.

114 Chinnaiyan P, Vallabhaneni G, Armstrong E, Huang SM, Harari PM. Modulation of radiation response by histone deacetylase inhibition. *Int J Radiat Oncol Biol Phys* 2005; **62**:223–229.

115 Richter K, Buchner J. Hsp90: chaperoning signal transduction. *J Cell Physiol* 2001; **188**:281–290.

116 Yang J, Yang JM, Iannone M, Shih WJ, Lin Y, Hait WN. Disruption of the EF-2 kinase/Hsp90 protein complex: a possible mechanism to inhibit glioblastoma by geldanamycin. *Cancer Res* 2001; **61**:4010–4016.

117 Bull EE, Dote H, Brady KJ, Burgan WE, Carter DJ, Cerra MA, *et al.* Enhanced tumor cell radiosensitivity and abrogation of G2 and S phase arrest by the Hsp90 inhibitor 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin. *Clin Cancer Res* 2004; **10**:8077–8084.

118 Marx GM, Pavlakis N, McCowatt S, Boyle FM, Levi JA, Bell DR, *et al.* Phase II study of thalidomide in the treatment of recurrent glioblastoma multiforme. *J Neurooncol* 2001; **54**:31–38.

119 Fine HA, Wen PY, Maher EA, Viscosi E, Batchelor T, Lakhani N, *et al.* Phase II trial of thalidomide and carmustine for patients with recurrent high-grade gliomas. *J Clin Oncol* 2003; **21**:2299–2304.

120 Baumann F, Bjeljac M, Kollias SS, Baumert BG, Brandner S, Rousson V, *et al.* Combined thalidomide and temozolomide treatment in patients with glioblastoma multiforme. *J Neurooncol* 2004; **67**:191–200.

121 Reardon DA, Quinn JA, Vredenburgh J, Rich JN, Gururangan S, Badruddoja M, *et al.* Phase II trial of irinotecan plus celecoxib in adults with recurrent malignant glioma. *Cancer* 2005; **103**:329.

122 Schlingensiepen KH, Schlingensiepen R, Steinbrecher A, Hau P, Bogdahn U, Fischer-Blass B, *et al.* Targeted tumor therapy with the TGF-beta2 antisense compound AP 12009. *Cytokine Growth Factor Rev* 2006; **17**:129–139.

123 Hjelmeland MD, Hjelmeland AB, Sathornsumetee S, Reese ED, Herbstreith MH, Laping NJ, *et al.* SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. *Mol Cancer Ther* 2004; **3**:737–745.

124 Uhl M, Aulwurm S, Wischhusen J, Weiler M, Ma JY, Almirez R, *et al.* SD-208, a novel transforming growth factor beta receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells *in vitro* and *in vivo*. *Cancer Res* 2004; **64**: 7954–7961.

125 Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. *Cancer Res* 2002; **62**:200–207.

126 Li B, Chang CM, Yuan M, McKenna WG, Shu HK. Resistance to small molecule inhibitors of epidermal growth factor receptor in malignant gliomas. *Cancer Res* 2003; **63**:7443–7450.

127 Rao RD, Mladek AC, Lamont JD, Goble JM, Erlichman C, James CD, *et al.* Disruption of parallel and converging signaling pathways contributes to the synergistic antitumor effects of simultaneous mTOR and EGFR inhibition in GBM cells. *Neoplasia* 2005; **7**:921–929.

128 Reardon DA, Quinn JA, Vredenburgh JJ, Gururangan S, Friedman AH, Desjardins A, *et al.* A phase I study of gefitinib plus rapamycin in recurrent glioblastoma multiforme. *Clin Cancer Res* 2006; **12**:860–868.

129 Shinohara ET, Cao C, Niermann K, Mu Y, Zeng F, Hallahan DE, *et al.* Enhanced radiation damage of tumor vasculature by mTOR inhibitors. *Oncogene* 2005; **24**:5414–5422.

130 Chakravarti A, Chakladar A, Delaney MA, Latham DE, Loeffler JS. The epidermal growth factor receptor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. *Cancer Res* 2002; **62**:4307–4315.

131 Damiano V, Melisi D, Bianco C, Raben D, Caputo R, Fontanini G, *et al.* Cooperative antitumor effect of multitargeted kinase inhibitor ZD6474 and ionizing radiation in glioblastoma. *Clin Cancer Res* 2005; **11**:5639–5644.

132 Williams KJ, Telfer BA, Brave S, Kendrew J, Whittaker L, Stratford IJ, *et al.* ZD6474, a potent inhibitor of vascular endothelial growth factor signaling, combined with radiotherapy: schedule-dependent enhancement of antitumor activity. *Clin Cancer Res* 2004; **10**:8587–8593.

133 Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, *et al.* Identification of human brain tumor initiating cells. *Nature* 2004; **432**: 396–401.

134 Staflin K, Honeth G, Kalliomaki S, Kjellman C, Edvardsen K, Lindvall M. Neural progenitor cell lines inhibit rat tumor growth *in vivo*. *Cancer Res* 2004; **64**:5347–5354.

135 Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, *et al.* Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 2005; **65**:3307–3318.