

Designer Therapies for Glioblastoma Multiforme

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Primary brain tumors account for less than 2% of all cancers in adults; however, they are often associated with neurologic morbidity and high mortality. Glioblastoma multiforme (GBM) has been a focus of new therapy development in neurooncology because it is the most common primary brain tumor in adults. Standard-of-care therapy for newly diagnosed GBM includes surgical resection, radiotherapy, and temozolomide, administered both during and after radiotherapy. However, most patients develop tumor recurrence or progression after this multimodality treatment. Repeat resection and stereotactic radiosurgery upon recurrence may improve outcome only in selected patients. Most salvage chemotherapies offer only palliation. Recent advances in our understanding of the molecular abnormalities of GBM have generated new therapeutic venues of molecularly targeted agents (designer drugs) against key components of cellular pathways critical for cancer initiation and maintenance. Such drugs may offer the potential advantage to increase therapeutic efficacy and decrease systemic toxicity compared with traditional cytotoxic agents. Nonetheless, first-generation targeted agents have failed to demonstrate survival benefits in unselected GBM patient populations. Several mechanisms of treatment failure of the first-generation designer drugs have been proposed, whereas new strategies have been developed to increase effectiveness of these agents. Here we will discuss the recent development and the strategies to optimize the effectiveness of designer therapy for GBM.

Key words: brain tumor; glioblastoma; glioma; targeted therapy; kinase inhibitors

Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumors in adults. The incidence of GBM is three per 100,000 person-years in the United States.¹ According to the World Health Organization (WHO) classification, GBM is characterized as grade IV astrocytoma with pathologic hallmarks of

necrosis and vascular proliferation. GBMs represent highly lethal cancers associated with significant morbidity and mortality. In a Swiss population-based study, the survival rate of patients with newly diagnosed GBM was approximately 18% at 1 year and only 3% at 2 years.² Despite available state-of-the-art multimodality treatments, the median survival of GBM patients is 12–15 months.³ Favorable prognostic factors include young age, absent or minimal neurological signs, complete surgical resection, and good performance status.⁴ Current standard-of-care therapies include surgery; radiation; and more recently, concurrent and adjuvant temozolomide. Recent elucidation

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of molecular abnormalities underlying glioma pathogenesis has led to several new therapeutic approaches, which include targeting specific oncogenic signaling elements by molecularly targeted therapy (designer drugs), immunotherapy, and gene therapy.⁵ Also, strategies to enhance delivery of therapeutic agents into the central nervous system, which include local polymer administration, convection-enhanced delivery (CED), and other new delivery systems such as nanoparticles, may increase therapeutic efficacy. In this review, we will discuss the recent advances in the development of new targeted therapy in GBM.

Current Standard-of-Care Treatment for GBM

Most GBM patients, after histological diagnosis, usually undergo multimodality treatments including surgical resection, radiation, and chemotherapy.⁶ Gross or near-total resection, if feasible, significantly improves survival.⁷ Radiation therapy has been the mainstay treatment for GBM for decades because it offers unequivocal survival benefit.⁸ Adjuvant chemotherapy had not demonstrated significant clinical benefit until recently when Stupp *et al.* reported that concurrent and adjuvant temozolomide (TMZ) significantly improved survival of GBM patients without degradation in quality of life in an international, multicentered trial.^{9,10} This pivotal phase III trial of 573 patients randomized to either radiation (external radiation therapy [XRT]) alone or radiation therapy with concurrent TMZ followed by monthly adjuvant TMZ for six cycles (XRT-TMZ/TMZ) demonstrated a 2-year survival rate of 24% for XRT-TMZ (75 mg/m² of body surface area/day for 42 consecutive days)/TMZ (150–200 mg/m²/day for 5 days every 28-day cycle) group compared with 10% for the XRT group. The median survival was 14.6 months for the XRT-TMZ/TMZ group compared with 12.1 months for the XRT group.⁹ Patients with treatment failure after

XRT received TMZ, so the study was powered to specifically test the effects of chemoradiation rather than adjuvant chemotherapy. TMZ adds clinical benefit without significant impairment of patient quality of life.¹⁰ Also, a recent economic analysis in Europe has shown that although the TMZ cost is high, its costs per life-year gained are comparable to accepted first-line treatment with chemotherapy in patients with other cancers.¹¹ Therefore, TMZ administered concurrently during XRT and after XRT, using adjuvant monthly cycles, has become a new standard-of-care treatment for GBM. Although TMZ significantly prolongs survival for GBM patients, the degree of benefit is modest. More strategies to enhance the efficacy of this regimen are clearly needed. Alternative dosing schedules; extended length of therapy; delivery enhancement; addition of agents to prevent or rescue TMZ resistance; and combination of TMZ with other modalities such as targeted therapeutics, gene therapy, or immunotherapy may improve treatment efficacy.

Most GBM patients develop recurrence or progression after the current standard treatments.¹² Surgical re-resection may increase survival in selected patients with recurrent GBM, mostly in symptomatic patients with large mass effect.¹² Locoregional treatments such as chemotherapy wafer,¹³ stereotactic irradiation,¹⁴ radioimmunoconjugates,¹⁵ and conjugated biological toxins¹⁶ may limit systemic toxicity and improve local tumor control because most GBMs recur within 2–3 cm of the primary tumor or resection site.¹⁷ These new locoregional treatments are currently under clinical investigation.

Unfortunately, available salvage chemotherapies after progression are ineffective, with “successes” demonstrating a 6-month progression-free survival (PFS-6) rate of 15% for GBM.¹⁸ PFS-6 has recently become a more widely acceptable primary endpoint for phase II trials in malignant glioma because it correlates with overall survival.^{19,20} Because of the lack of effective chemotherapies,

new therapies targeting underlying pathogenesis of malignant gliomas are obviously needed.

Genetic Alterations in GBM

GBM, like other cancers, exhibits characteristic malignant phenotypes, including self-sustained proliferation, resistance to apoptotic stimuli, evasion of external growth control and immunosurveillance, tissue invasion, and ability to form and sustain new blood vessels.⁵ GBM is genetically heterogeneous between patients and within tumors.^{21,22} Furthermore, evolving molecular aberrations from dynamic genetic instability are also characteristic for GBM. Nevertheless, frequent genetic alterations that maintain malignant phenotypes of tumors have been described. Most GBMs (90%) are diagnosed without antecedent lower-grade tumor—termed primary or *de novo* GBM, whereas secondary GBM has clinical evidence of transformation from lower-grade gliomas.²³ Low-grade astrocytomas (WHO grade II) often display disruption of tumor suppressor gene *TP53* and overexpression of platelet-derived growth factor (PDGF) ligands and receptors. In response to genotoxic stress, the *TP53* gene functions to induce cell cycle arrest, apoptosis, and DNA repair. Inactivation of *TP53* is associated with abnormal cell division and neoplastic transformation. Progression to anaplastic astrocytoma involves accumulation of other genetic alterations of associated cell cycle regulatory pathways, including deletion or mutations of cyclin-dependent kinase inhibitor p16^{INK4A}/CDKN2A or the retinoblastoma susceptibility locus 1 (pRB1), as well as amplification or overexpression of cyclin-dependent kinase (*CDK*)4/6 and human double minute 2 (*HDM2*). Transformation to GBM (i.e., secondary GBM) is associated with deletion of chromosome 10, which includes the tumor suppressor phosphatase and tensin homolog (*PTEN*). Primary GBMs tend to occur more often in older patients than sec-

ondary GBMs and primary GBMs share some genetic abnormalities with secondary GBMs such as loss of *PTEN*, deletion or mutation of cyclin-dependent kinase inhibitors p16^{INK4A} (which shares a locus with p14^{ARF} on chromosome 9), and amplification of *HDM2* or *CDK4*. However, additional molecular changes distinguish primary and secondary GBMs. Epidermal growth factor receptor (*EGFR*) amplification is more common in primary GBMs, whereas *TP53* loss is a genetic hallmark of low-grade astrocytoma and secondary GBMs (Fig. 1). Transcriptional profiling has demonstrated common and differential gene expression between primary and secondary GBMs.²⁴ Primary GBM-associated genes involve stromal and mesenchymal stem cell-like properties, whereas secondary GBM-associated genes commonly involve mitotic cell cycle components.

Some of these genetic abnormalities deregulate signal transduction pathways, a communication network of regulatory molecules within the cell, controlling cellular processes contributing to normal homeostasis and malignancy. For example, amplification or mutation of EGFR can increase activity of the RAS mitogen-activated protein kinase (MAPK) and phosphatidylinositide-3-kinase (PI3K)/AKT pathways. PI3K/AKT overactivity may also result from loss of PTEN, a negative regulator of PI3K function. Understanding these molecular and genetic abnormalities has led to a rational development of molecularly targeted (designer) therapies in GBM.

Designer Drugs Targeting Signal Transduction Pathway

Signal transduction pathways are regulated by several growth factors, hormones, and cytokines. Most receptors for growth factor pathways (e.g., EGF, PDGF, and vascular endothelial growth factor [VEGF]) are associated with tyrosine kinase activity and therefore share

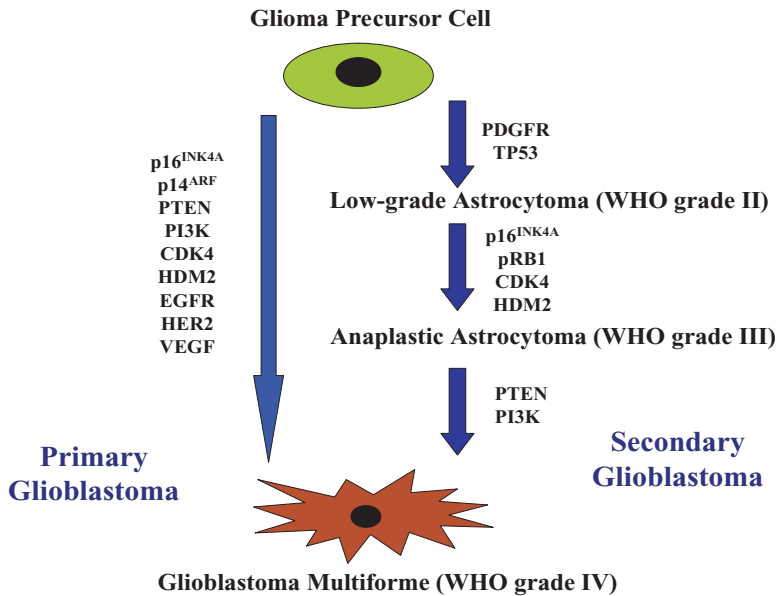


Figure 1. Genetic alterations in glioblastoma. Secondary GBM can develop from malignant transformation of lower-grade astrocytomas (low-grade astrocytoma [WHO grade II] or anaplastic astrocytoma [WHO grade III]), whereas the more common type, primary GBM, develops without antecedent lower-grade tumors. Genetic analyses reveal common and differential molecular aberrations between primary and secondary GBMs. No single genetic mutation represents malignant astrocytomas, indicating the inherent genetic heterogeneity of these tumors. Therapeutic agents targeting only single genetic/molecular pathways are less likely to achieve tumor control in a broad range of patients.

common mechanisms of pathway activation. Overexpression or mutations of receptors and intracellular downstream effectors have been identified in malignant gliomas, leading to constitutive activation of signaling pathways, resulting in uncontrolled cellular proliferation, survival, invasion, and secretion of angiogenic factors (Fig. 2). New treatments have been designed to target molecules in these signaling pathways with the goal to increase specific efficacy and minimize toxicity.²⁵ Monoclonal antibodies and low-molecular-weight inhibitors are among common targeted therapeutics used in cancer. Monoclonal antibodies are multivalent proteins engineered to have high selectivity and affinity to antigenic epitopes. In brain tumors, most monoclonal antibodies are delivered locally to tumor or resection cavity because systemic administration may not achieve adequate delivery owing to restriction by the blood–

brain barrier. Modulation of blood–brain barrier integrity may overcome this challenge. Also, monoclonal antibodies that can function on the abluminal side of blood vessels (such as a neutralizing VEGF antibody, bevacizumab) without a need to traverse the blood–brain barrier may be effective in the treatment of brain tumors.

Low-molecular-weight kinase inhibitors are often ATP mimetics that display affinity for the ATP binding site in the kinase domains of growth factor receptors and intracellular signaling elements. The specific targeting of single kinases has proven challenging because the ATP site is highly conserved in the kinase genes. The initial desire to limit off-target effects of these inhibitors has been tempered by the success of less selective inhibitors (previously called “dirty” but now retermed “multiselective”) in clinical trials (e.g., sunitinib

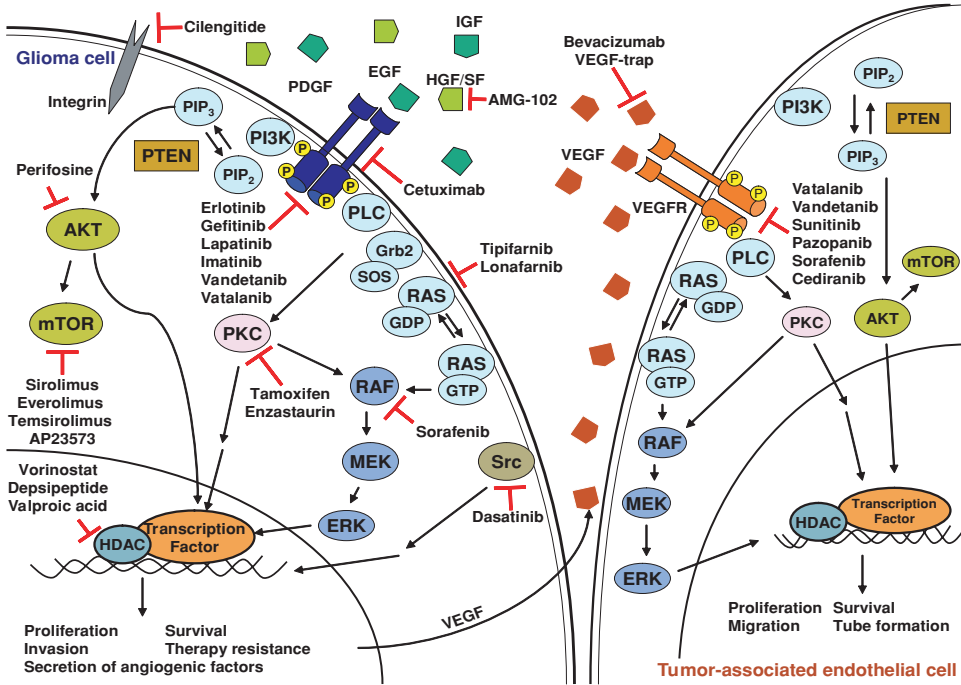


Figure 2. Signal transduction pathways and designer drugs. Glioblastoma cells and associated endothelial cells often have constitutive activation of the pathways of several growth factor receptors such as EGFR, VEGFR, and platelet-derived growth factor receptor (PDGFR). Each growth factor family consists of several members for which cognate receptors are transmembrane glycoproteins associated with protein tyrosine kinase activity. Ligand binding to receptors induces receptor dimerization and phosphorylation (P). This receptor activation permits the binding of adaptor proteins such as growth factor receptor-bound 2 (Grb2)/son of sevenless (SOS) and induces the activity of many intracellular signal transduction pathways that regulate gene transcription of essential cellular proteins contributing to malignancy. Several points in these cascades are the targets of therapies in development for malignant glioma, some of which are shown. Signaling molecules might include RAS, RAF, mitogen-activated protein extracellular-regulated kinase (MEK), extracellular regulated kinase (ERK; also termed mitogen-activated protein kinase [MAPK]), phosphatidylinositide-3-kinase (PI3K), AKT, mammalian target of rapamycin (mTOR), and protein kinase C (PKC). Several points in these cascades are the targets of therapies in development for malignant gliomas, some of which are shown. GDP, guanine diphosphate; GTP, guanine triphosphate; HDAC, histone deacetylase; PIP₂, phosphatidylinositol (4,5) bisphosphate; PIP₃, phosphatidylinositol (3,4,5) trisphosphate; PLC, phospholipase C; PTEN, phosphatase and tensin homolog. (Adapted from Sathornsumetee *et al.*⁵)

and sorafenib). Many low-molecular-weight kinase inhibitors have undergone preclinical and clinical investigation in brain tumors, mostly malignant gliomas. Because of their small size, these low-molecular-weight inhibitors might have advantage for central nervous system (CNS) delivery. However, several other factors such as physiological variables; polarity of drugs; and active efflux transporter at the blood–brain, blood–cerebrospinal fluid, or blood–tumor barrier might limit CNS and sub-

sequent tumor delivery. Strategies to enhance delivery of kinase inhibitors are needed.

Inhibition of Growth Factor Signaling Pathways

Relevant growth factor pathways in malignant gliomas include EGF, PDGF, VEGF, insulin-like growth factor (IGF), fibroblast growth factor, and hepatocyte growth factor/scatter factor (HGF/SF). In GBM, several

growth factor receptors (e.g., EGFR, VEGFR, PDGFR) are overexpressed or mutated, leading to activation of downstream signaling pathways with subsequent stimulation of proliferation, survival, invasion, and secretion of angiogenic factors. Kinase inhibitors and monoclonal antibodies of these ligands or receptors have been developed in clinical trials for malignant glioma.

Epidermal Growth Factor Pathway

EGFR is amplified in approximately half of GBMs and is overexpressed in many malignant gliomas independent of amplification status.²⁶ Also, the frequent overexpression of several EGFR mutants, including a variant with loss of exons 2–7 (EGFRvIII) resulting in the loss of extracellular ligand binding but constitutive activation, suggests that EGFR is a key factor in gliomagenesis and provides a rationale for the use of EGFR targeted therapies in these patients.²⁷ Two kinase inhibitors of EGFR, erlotinib (Tarceva, OSI-774; Genentech, South San Francisco, CA) and gefitinib (Iressa, ZD1839; AstraZeneca, Wilmington, DE), have been evaluated in malignant gliomas. In the first phase II trial of gefitinib for recurrent GBM, the median event-free survival was only 8.1 weeks, and no radiographic responses were observed, although nine of the 53 patients (17%) remained event free for at least 6 months.²⁸ Another phase II trial from Italy has confirmed the ineffectiveness of gefitinib in high-grade glioma patients.²⁹ In a published phase I trial, erlotinib as monotherapy or in combination with temozolomide demonstrated a 14% partial response (PR; a >50% decrease in maximal area on radiographic evaluation) rate and a PFS-6 of 11%.³⁰ Other phase II trials of erlotinib have demonstrated a PR rate of 6%–25% with modest effect on progression-free or overall survival rates.^{31,32} Therefore, erlotinib appears to be more effective against malignant gliomas than gefitinib for radiographic response rate, but both have no clear effect on survival. Small fractions of lung cancer patients display remarkable responses to EGFR

inhibitors that are associated with mutations in the kinase regions of EGFR that create a constitutively active receptor kinase.³³ These radiographic responses and kinase mutations have not been detected in glioma patients.^{34–36} However, two retrospective studies demonstrated that high expression of wild-type EGFR and low levels of phosphorylated Akt in one study³⁷ and coexpression of EGFRvIII and wild-type PTEN in another study³⁸ were associated with increased radiographic response to EGFR kinase inhibitors (erlotinib and gefitinib). In contrast, a relatively large (110 patients) phase II randomized study of erlotinib versus temozolomide or carmustine in recurrent malignant gliomas conducted by the European Organization for Research and Treatment of Cancer (EORTC) demonstrated no radiographic response or survival benefit of erlotinib, and the expression of EGFR, EGFRvIII, or PTEN was not correlated with survival advantage.³⁹ In fact, coexpression of EGFRvIII and wild-type PTEN was associated with decreased overall and progression-free survival.³⁹ The discrepancy observed among these biomarker studies may derive from different substrates and techniques of biomarker assessment, small sample size, and varied radiographic response criteria. Clearly, prospective validation with a standardized biomarker assay among different laboratories is required to resolve this controversy. Until then, routine use of gefitinib or erlotinib, even in patients with EGFRvIII and wild-type PTEN coexpression, should be approached conservatively.

Irreversible EGFR inhibitors have demonstrated superior efficacy in preclinical studies against cancer harboring EGFRvIII to reversible EGFR kinase inhibitors such as gefitinib and erlotinib.⁴⁰ Clinical development of irreversible EGFR kinase inhibitors in malignant gliomas with EGFRvIII mutation appears warranted. In addition to small-molecule inhibitors, a monoclonal antibody against EGFR, cetuximab (Erbix; Imclone Systems, New York, NY), has demonstrated preclinical antitumor activity as a single agent and combinatorial

benefit with radiation against EGFR-amplified GBM.⁴¹ Despite the concern of limited CNS delivery, as described in the preceding, cetuximab is undergoing clinical evaluation in patients with recurrent GBMs either alone or in combination with other targeted therapies such as bevacizumab.⁴²

PDGF Pathway

PDGF signaling is important for growth and angiogenesis of gliomas. Infusion of PDGF in rodent brains induces neural stem cells to form glioma-like growths.⁴³ Imatinib mesylate (Gleevec, STI571; Novartis Pharmaceuticals, East Hanover, NJ), an inhibitor of PDGFR, c-kit, and bcr-abl kinases, exhibited antiglioma activity in preclinical studies.⁴⁴ However, imatinib monotherapy failed to demonstrate benefit for malignant glioma patients in several phase I/II trials.⁴⁵ Nonetheless, imatinib mesylate in combination with hydroxyurea has demonstrated promising, albeit modest, antitumor activity in a patient series,⁴⁶ which was subsequently confirmed by a phase II study.⁴⁷ In this trial of 33 patients with recurrent GBMs, the radiographic response rate was 9% with a PFS-6 of 27%. Another study confirmed the antitumor activity of this regimen in recurrent grade 3 malignant glioma patients.⁴⁸ The treatment combination was well tolerated. The mechanism contributing to combinatorial benefit of imatinib and hydroxyurea remains to be elucidated. Because of the encouraging results of imatinib mesylate plus hydroxyurea, several combinations of imatinib mesylate with other chemotherapies such as temozolomide are under clinical investigation. A recently published phase I trial of imatinib mesylate in combination with temozolomide has revealed the safety and tolerability with some hints of activity.⁴⁹ Further evaluation in larger studies is needed.

VEGF Pathway

Angiogenesis, the creation of new blood vessels from preexistent blood vessels, is a pathologic hallmark of cancer. For tumor growth beyond approximately 2 mm³, a new network of

blood vessels must be constructed for nutrient and oxygen supply (the “angiogenic switch”).⁵⁰ This angiogenesis dependence of tumor relative to normal organs provides an opportunity to develop specific tumor-targeted therapy. Expression of VEGF, a key regulator of tumor angiogenesis, increases with the grade of gliomas, whereas microvessel density is associated with poor prognosis in glioma patients.^{51,52} The VEGF pathway can be targeted at the level of VEGF ligands or at the level of VEGFR.⁵³

Targeting VEGF Ligands

Targeting the VEGF pathway has been one of the most exciting focuses in the treatment of malignant gliomas in the past few years because unprecedented radiographic response and survival benefit were observed with a VEGF-targeted agent, bevacizumab, in combination with irinotecan.^{54,55} Bevacizumab (Avastin; Genentech), a recombinant human neutralizing monoclonal antibody of VEGF, is the first U.S. Food and Drug Administration–approved antiangiogenic agent in cancer treatment. Preclinical studies of A4.6.1, a murine counterpart of bevacizumab, reduced tumor vascularity, enhanced tumor apoptosis, and prolonged survival in preclinical studies with a rat intracranial C6 glioma model.⁵⁶ A4.6.1 also offered a synergistic effect with radiation therapy in GBM xenografts.⁵⁷ The antitumor mechanism of bevacizumab is unclear. Changes in vascular structure and function have been 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 because of transient improved vascular function (“forced normalization”), implying that the most effective use of anti-VEGF therapy may be in combination with chemotherapy.^{58,59} Bevacizumab has demonstrated encouraging antitumor activity in combination with topoisomerase I inhibitor, irinotecan (Camptosar, CPT-11; Pfizer, New York, NY) in an anecdotal series, which was

confirmed in a phase II trial at Duke University.⁵⁴ This combination demonstrated a remarkable radiographic response rate of 63% with a PFS-6 of 32% for GBM and 61% for recurrent anaplastic gliomas. This regimen was generally well tolerated, with similar side effects to the use of bevacizumab in other cancers (e.g., hypertension, changes in renal function). The encouraging radiographic response rates detected in this initial phase prompted an expansion to include a total of 68 patients with recurrent malignant gliomas. The PFS-6 for all 68 patients was 43% for recurrent GBM and 61% for recurrent anaplastic gliomas.⁵⁵ There was only one intracerebral hemorrhage that occurred after 1 year of treatment. A few patients developed venous thromboembolism and one patient had an arterial ischemic stroke. Because irinotecan monotherapy was not associated with survival benefit in several prior clinical trials, the contribution of irinotecan to antitumor activity of this regimen is under investigation in a phase II study of bevacizumab versus bevacizumab plus irinotecan.⁶⁰ Preliminary results suggest that patients enjoy greater benefit from the combination of bevacizumab and irinotecan than from bevacizumab alone and support the use of anti-VEGF therapy in combination with cytotoxic agents.⁶⁰ Several clinical trials of bevacizumab in combination with radiation therapy, chemotherapy, or other targeted agents are ongoing.

The radiographic response observed by contrast-enhanced magnetic resonance imaging with bevacizumab treatment does not necessarily translate into overall survival benefit because targeting VEGF alters vascular permeability without necessarily altering the tumor directly, so several strategies such as metabolic imaging (positron emission tomography [PET]) and tumor immunohistochemical profiling have been exploited to define predictive biomarkers. [¹⁸F]thymidine PET, an imaging biomarker of cell proliferation, was assessed in 21 malignant glioma patients treated with bevacizumab and irinotecan.⁶¹ Patients with

greater than 25% reduction in [¹⁸F]thymidine uptake on PET imaging (“metabolic response”) at 1–2 weeks and 6 weeks after treatment initiation experienced improved survival. More recently, we have reported that tumor expression of VEGF, a molecular target of bevacizumab, at the time of original diagnosis assessed by immunohistochemistry was associated with increased likelihood of radiographic response but not survival benefit in malignant astrocytoma patients treated with bevacizumab and irinotecan.⁶² Tumor hypoxia as measured by high carbonic anhydrase-IX expression was associated with poor survival outcome in this patient population.⁶² Prospective validation of both imaging and tissue biomarkers for bevacizumab in malignant gliomas is warranted.

In patients with recurrence after initial response to bevacizumab plus single chemotherapy, continuing bevacizumab and changing the chemotherapy agent provide disease control only in a few patients.⁶³ Also, bevacizumab may alter the recurrence pattern of malignant gliomas by suppressing enhancing tumor recurrence more effectively than it suppresses nonenhancing, infiltrative tumor growth.⁶³ In addition to its activity in recurrent malignant gliomas, bevacizumab was also evaluated in newly diagnosed GBM patients as an up-front treatment with radiotherapy and temozolomide.⁶⁴ This phase II pilot study demonstrated safety and acceptable toxicities in the first 10 patients (planned enrollment of 70 patients) with encouraging antitumor activity.

Another agent that inhibits VEGF by blocking ligand–receptor binding is VEGF-trap (Regeneron, Tarrytown, NY).⁶⁵ VEGF-trap is a potent soluble decoy receptor of VEGF that effectively suppresses tumor growth and angiogenesis in preclinical cancer models.⁶⁵ VEGF-trap has antiglioma activity as high-dose monotherapy, but it offered combinatorial benefit even at low dose with radiation therapy in a subcutaneous human GBM xenograft model.⁶⁶ A clinical trial of VEGF-trap in recurrent malignant gliomas is ongoing.

Targeting VEGF Receptor

Preclinical evaluation of VEGFR inhibition by both monoclonal antibody and kinase inhibitor have demonstrated efficacy against malignant gliomas. Vatalanib (PTK787/ZK222584; Novartis), a kinase inhibitor of VEGFR and PDGFR, has demonstrated modest efficacy in multicentered phase I/II trials either alone or in combination with chemotherapy.^{67,68} Recently, a phase II trial of cediranib (AZD2171; AstraZeneca), a pan-VEGFR inhibitor, has demonstrated encouraging antiangiogenic efficacy in GBM patients with radiographic response rate of 56% and APF (alive and progression-free) at 6 months of 27.6%.⁶⁹ Significant increases in plasma VEGF and in placental growth factor and a decrease in soluble VEGFR-2 were observed during the treatment. In patients who developed disease progression, plasma angiogenic profile changes with not only decrease in placental growth factor and increase in soluble VEGFR-2 levels but also increases in viable circulating endothelial cells, basic fibroblast growth factor, and stromal-derived factor 1 α levels. Also, dynamic contrast-enhanced magnetic resonance imaging along with diffusion-weighted and tractographic imaging were used to monitor the “normalization” phenomenon and clinical response in GBM patients treated with cediranib.⁶⁹

IGF Receptor

IGF signaling is important in regulating cell growth and proliferation.⁷⁰ IGF-1R, a receptor tyrosine kinase, has been a prominent target for cancer therapeutics. A preclinical study demonstrated that an EGFR kinase inhibitor-resistant GBM cell line had an upregulation of IGF-1R, preferentially activating the PI3K pathway, resulting in proliferative, antiapoptotic, and proinvasive potentials.⁷¹ Targeting this resistant cell line with a combination of EGFR and IGF-1R inhibitors enhanced spontaneous and radiation-induced apoptosis and reduced tumor invasion. Several therapeutic

agents against IGF-1R have been developed in a preclinical phase.⁷²

HGF Pathway

HGF/SF is upregulated in many human cancers, including GBM.⁷³ HGF and its cognate receptor tyrosine kinase, c-met, are expressed on glioma cells, suggesting autocrine and paracrine loops of activation. HGF/SF-met signaling is associated with tumor cell proliferation, invasion, and angiogenesis, and expression of pathway components increases with malignant progression.⁷³ Neutralizing monoclonal antibodies to HGF/SF as monotherapy and in combination with temozolomide have demonstrated antitumor activity in both subcutaneous and orthotopic malignant glioma xenograft models.⁷⁴⁻⁷⁹ A multicentered phase II trial of AMG-102 (Amgen, Thousand Oaks, CA), an HGF/SF monoclonal antibody, is ongoing in advanced malignant glioma.

Transforming Growth Factor β Pathway

Transforming growth factor β (TGF- β) is a multifunctional cytokine secreted from glioma cells to regulate cell motility, invasion, immune surveillance, and angiogenesis. Upon binding to TGF- β ligand, TGF- β receptors (type I and II) become heterodimerized and phosphorylated to activate downstream effectors in the SMAD family and promote gene transcription. High TGF- β -SMAD activity levels are present in aggressive, highly proliferative gliomas and confer poor prognosis in patients.⁸⁰ A phase I/II trial of intratumoral AP 12009, an antisense oligodeoxynucleotide specific to TGF- β 2, demonstrated good tolerability and promising antitumor activity with median survival of 47 weeks for recurrent GBM.⁸¹ Several low-molecular-weight inhibitors of TGF- β receptors have demonstrated antitumor efficacy and induction of antitumor immunity in preclinical models of gliomas.^{82,83} These agents might be evaluated in clinical trials as monotherapies or in combination with other treatment modalities

such as chemotherapy or radiation in patients with malignant gliomas.

Inhibition of Intracellular Effectors

After growth factor receptor activation, effector molecules such as RAS, PI3K, and phospholipase C are recruited to the cell membrane.⁸⁴ Many gliomas are associated with either activation of these effector molecules or inactivating mutations of the negative regulators of these kinases such as PTEN in the PI3K pathway. Sequential activation by phosphorylation of intracellular effectors along signal transduction pathways relays important information to regulate cellular processes contributing to malignancy. Crucial intracellular mediators in oncogenic pathways include RAF, mitogen-activated protein extracellular-regulated kinase (MEK), extracellular-regulated kinase (ERK; also termed MAPK), AKT, and mammalian target of rapamycin (mTOR). A variety of designer inhibitors of these intracellular effectors have been developed in preclinical and clinical studies of malignant gliomas.

RAS–RAF–MEK–ERK Pathways

RAS encodes small GTP-binding proteins that regulate many cellular functions such as proliferation, differentiation, cytoskeletal organization, protein trafficking, and the secretion of angiogenic factors.⁸⁵ Gliomas rarely contain oncogenic RAS mutations; however, they often have high RAS activity due to mutations or overexpression of upstream growth factor receptors. RAS proteins, like many proteins, undergo posttranslational modification with the addition of lipids to permit membrane localization. This process, called prenylation, may involve the addition of either farnesyl or geranylgeranyl groups. Prenylation is the rate-limiting step in RAS maturation; therefore, several farnesyltransferase inhibitors have undergone clinical evaluation as a RAS targeted therapy. Two farnesyltransferase inhibitors, tipifarnib (Zarnestra, R115777; Johnson & Johnson, New Brunswick, NJ) and lona-

farnib (Sarasar, SCH66336; Schering-Plough, Berkeley Heights, NJ), have been developed. A phase I/II study of tipifarnib in recurrent malignant gliomas demonstrated a PFS-6 of 9% in recurrent WHO grade III gliomas and 12% in recurrent GBMs.⁸⁶ In a phase I trial of temozolomide plus lonafarnib, 27% of patients with prior temozolomide failure had a PR, and the estimated PFS-6 was 33%.⁸⁷ Downstream from RAS is the RAF–MEK–ERK pathway, which regulates mainly cell proliferation. Activation of ERK is associated with poor outcome in GBM patients.⁸⁸ Thus, targeting the RAF–MEK–ERK pathway may be effective in malignant glioma. A preclinical study of a RAF/VEGFR inhibitor, AAL881 (Novartis), has demonstrated significant *in vitro* and *in vivo* antiglioma activity.⁸⁹ Clinical trials of sorafenib (Nexavar; Bayer, West Haven, CT, and Onyx, CA), another inhibitor of RAF/VEGFR, in combination with several other targeted agents are ongoing.

PI3K–AKT–mTOR Pathways

PI3K pathways regulate several malignant phenotypes including antiapoptosis, cell growth, proliferation, and invasion.⁹⁰ Activation of PI3K pathways is associated with poor prognosis in glioma patients.⁹¹ Loss of *PTEN* is a common genetic feature in GBM that leads to constitutive activation of the PI3K pathway. Activated PI3K phosphorylates several downstream effectors, including AKT. Inhibitors of PI3K and AKT have undergone preclinical evaluation with encouraging results.^{92,93} Perifosine (Keryx Biopharmaceuticals, New York, NY), an oral AKT and AMPK inhibitor, is undergoing clinical evaluation in malignant gliomas.⁹³ Also, preclinical studies have demonstrated antitumor efficacy of several integrin-linked kinase inhibitors by AKT inhibition.⁹⁴

mTOR is downstream from AKT and can be activated by not only AKT but also RAS pathways. Rapamycin (Sirolimus, Rapamune; Wyeth, Collegeville, PA) and its synthesized analogues, temsirolimus (CCI-779,

Wyeth), everolimus (RAD001, Novartis), and AP23573 (Ariad Pharmaceuticals, Cambridge, MA), have been evaluated in clinical trials of malignant gliomas. Two recent phase II studies of temsirolimus monotherapy in recurrent GBMs demonstrated varied radiographic improvement without survival benefit as measured by a PFS-6 of only 2.5%–7.8%.^{95,96} Taken together, rapamycin analogues might not be sufficient for tumor control as monotherapies. One plausible explanation may be the selective inhibition of only the mTOR1C complex without affecting mTOR2C complexes that regulate cell polarity, growth, and invasion.⁹⁷ Targeted deletion of entire mTOR activities by small interfering RNA can rescue the sensitivity of rapamycin-resistant cell lines to rapamycin.⁹⁸ Alternatively, blocking mTOR may stimulate other signaling elements critical for cell survival. Few preclinical studies demonstrated that inhibition of mTOR can stimulate kinase activity of its immediate upstream effector, AKT, which may decrease the anti-tumor efficacy.⁹⁹ PI-103, a novel inhibitor of both PI3K and mTOR, has shown promising activity in both *in vitro* and *in vivo* models of malignant gliomas, partly due to blocking activated PI3K/AKT induced by mTOR inhibition.¹⁰⁰

Protein Kinase C Pathways

Protein kinase C (PKC) is a serine/threonine kinase that regulates cell proliferation, invasion, and angiogenesis. The PKC- β inhibitor with activity against glycogen synthase kinase 3 β , enzastaurin (LY317615; Eli-Lilly, Indianapolis, IN), has demonstrated activity against glioma xenografts as both monotherapy and synergism with radiotherapy.^{101,102} A phase II trial of enzastaurin in recurrent malignant gliomas yielded a promising 29% radiographic response rate.¹⁰³ However, a multicentered phase III trial of enzastaurin versus lomustine was prematurely terminated because of failure to achieve a survival benefit in an interim analysis.

Miscellaneous

Several other molecular targets are candidates for development of novel therapy in malignant astrocytoma. The src kinase is a multifunctional, intracellular tyrosine kinase that regulates cellular proliferation, survival, motility, and angiogenesis.¹⁰⁴ Dasatinib (BMS-354825; Bristol-Myers Squibb, New York, NY), a dual inhibitor of src and bcr-abl kinases, is undergoing clinical evaluation in malignant glioma as both monotherapy and in combination with erlotinib. Focal adhesion kinase (FAK) is a tyrosine kinase involved in cancer invasion and metastasis. These kinases are dynamic intracellular proteins that link the extracellular matrix to the cell cytoskeleton through integrins. Higher expression of FAK correlates with glioma grade. FAK inhibitors have demonstrated preclinical efficacy in malignant gliomas.¹⁰⁵ Thus, development of these inhibitors in clinic may be warranted.

Integrins are cell adhesion molecules important in glioma cell migration and angiogenesis.¹⁰⁶ Cilengitide (EMD121974; EMD Pharmaceuticals, Durham, NC), an intravenous inhibitor of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, demonstrated preclinical efficacy against malignant glioma. A phase I trial of cilengitide in recurrent malignant gliomas by the New Approaches to Brain Tumor Therapy group has been completed with no dose-limiting toxicities and an encouraging 10% radiographic response rate.¹⁰⁷ Preliminary results of a phase I/II trial of cilengitide with temozolomide and radiation therapy followed by cilengitide/temozolomide in newly diagnosed GBM have been encouraging with a PFS-6 of 65%. A phase II trial of cilengitide in recurrent GBM has also been completed.

Histone deacetylase (HDAC) inhibitors induce cell cycle arrest and apoptosis in cancer cells.¹⁰⁸ Pretreatment with an HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA, Vorinostat; Aton Pharma, Tarrytown, NY), sensitizes glioma cells to radiation and

chemotherapy.^{109,110} Clinical trials of vorinostat as monotherapy or in combination with temozolomide in malignant glioma are ongoing. Another HDAC inhibitor, depsipeptide (FK228; Gloucester Pharmaceuticals, Cambridge, MA), demonstrated preclinical efficacy in GBM. A phase I/II study of depsipeptide in recurrent malignant gliomas is ongoing by the North American Brain Tumor Consortium.

The ubiquitin–proteasome system is important in regulating cell cycle proteins to balance cell proliferation and apoptosis.¹¹¹ Disruption of the temporal degradation of these regulatory molecules by proteasome inhibitors can induce cell growth arrest and apoptosis. A proteasome inhibitor, bortezomib (Velcade, PS-341; Millennium Pharmaceuticals, Cambridge, MA), induced cell cycle arrest and apoptosis in glioma cell lines.¹¹² Several clinical trials of bortezomib in combination with temozolomide or other targeted agents such as vorinostat, bevacizumab, or tamoxifen are ongoing¹¹³ or planned.

Strategies to Improve Therapeutic Efficacy

Current targeted therapies in malignant gliomas have been associated with various response rates and modest to no survival benefits (Table 1). Several strategies have been developed to improve the effectiveness of targeted agents for this devastating cancer (Fig. 3). Among these may include new target identification, drug delivery enhancement, multitargeted inhibitors, new treatment combinations, biomarker identification, and improved preclinical and clinical designs.

Identification of New Targets

Several inhibitors of new targets have emerged in preclinical or early clinical development for the treatment of cancers. Cell cycle regulators such as cyclin-dependent kinase inhibitors, checkpoint kinase inhibitors, aurora

kinase¹¹⁴ and polo-like kinase inhibitors, and mitotic kinesin inhibitors have been evaluated in various hematologic and solid malignancies. These agents may also be candidates for glioma treatment. Preclinical studies in malignant gliomas have elucidated many other potential targets, which may include cannabinoid receptors,¹¹⁵ telomerase, myc, and signal transducer and activator of transcription 3.¹¹⁶ New gene genome analyses such as those found in the National Cancer Institute, the *Cancer Genome Atlas*, Genomic Identification of Significant Targets in Cancer, system biology, and bioinformatics may identify new therapeutic targets.^{117,118}

Brain tumors, like all cancers, are essentially aberrant organ systems with heterogeneous cell types that include not only neoplastic cells but also endothelial cells, inflammatory cells, and invading astrocytes. The neoplastic compartment displays cells with a diversity of differentiation markers. More than a century ago, these observations led to the hypothesis that cancers contain a subset of relatively undifferentiated cells. In parallel to the function of tissue-specific stem cells in development and regeneration, neoplastic cells that display a stem cell–like phenotype may be important in tumor initiation and maintenance. Thus, cancer stem cells (also called tumor-initiating cells or tumor-propagating cells) have been defined by sustained self-renewal and the ability to generate the diversity of tumor cell types present in cancers. These functional assays have limited the ability to study cancer stem cells prospectively until the recent development of cell surface markers that can be used to enrich or deplete cancer stem cells. It is essential to the understanding of the cancer stem cell hypothesis that the presence of cancer stem cells does not require a stem cell of origin. Recent identification of cancer stem cells in solid malignancies, including glioblastoma, has generated a change of thought for cancer research, including the therapeutic discovery.¹¹⁹ Glioblastoma stem cells have contributed to malignant properties, including angiogenesis and therapeutic

TABLE 1. Molecular Targeted Therapies Disrupting Signal Transduction Pathways in Malignant Gliomas

Target(s)	Agent(s)	Phase	Results/Status	
Growth factor receptors EGFR	Gefitinib	II	Recurrent GBM (1st relapse): no radiographic response; PFS-6: 17%	
	Erlotinib (\pm TMZ)	I/II	Recurrent MG: 14% PR; PFS-6: 11%	
	Erlotinib	I/II	Recurrent MG: 6%–25% PR; PFS-6 10%–20%	
	Erlotinib + RT	I	Newly diagnosed GBM: MTD—not reached; median TTP: 26 weeks	
	Erlotinib (+TMZ, bevacizumab)	II	Newly diagnosed GBM—stable after radiation therapy: ongoing	
	Cetuximab	II	Recurrent GBM: ongoing	
	Cetuximab (+TMZ/RT)	I/II	Newly diagnosed GBM: ongoing	
	Cetuximab (+bevacizumab/irinotecan)	II	Recurrent GBM: ongoing	
	VEGF	Bevacizumab + irinotecan	II	Recurrent MG: 63% CR + PR PFS-6 GBM 43%; AA/AO 61%
		Bevacizumab versus bevacizumab + irinotecan	II	Recurrent GBM: completed
Bevacizumab + erlotinib (EGFR inhibitor)		II	Recurrent MG: ongoing	
Bevacizumab + metronomic TMZ		II	Recurrent MG: ongoing	
Bevacizumab plus etoposide		II	Recurrent MG: ongoing	
Bevacizumab plus XRT		II	Newly diagnosed GBM: ongoing	
Bevacizumab plus XRT and TMZ		II	Newly diagnosed GBM: ongoing	
VEGF trap		II	Recurrent MG: ongoing	
Vatalanib (\pm temozolomide or lomustine)		I/II	Recurrent GBM: 4% PR; 66% SD; TTP: 12–16 weeks	
Pazopanib (+lapatinib-HER1/2 inhibitor)		I	Recurrent MG: ongoing	
HGF/SF	Cediranib (AZD2171)	II	Recurrent GBM: 56% PR; APF-6: 27.6%	
	AMG-102	II	Advanced MG: ongoing	
PDGFR	Imatinib mesylate	II	Recurrent GBM: PFS-6: 3% Recurrent AA: PFS-6: 10%	
	Imatinib mesylate + hydroxyurea	II	Recurrent GBM: 9% PR; 42% SD PFS-6: 27%	
	Imatinib mesylate, hydroxyurea, and vatalanib	I	Recurrent MG: ongoing	
Intracellular effectors RAS (Farnesyltransferase)	Tipifarnib	I/II	Recurrent GBM: PFS-6: 12% Recurrent AA: PFS-6: 9%	
	Lonafarnib (+TMZ)	I	Recurrent GBM: 27% PR; PFS-6: 33%	
RAF (+VEGFR-2)	Sorafenib (+ erlotinib, tipifarnib, or temsirolimus)	I/II	Recurrent MG: ongoing	
	Sorafenib (+erlotinib)	II	Recurrent/progressive GBM	

Continued

TABLE 1. *Continued*

Target(s)	Agent(s)	Phase	Results/Status
AKT mTOR	Perifosine	II	Recurrent MG: ongoing
	Sirolimus (+gefitinib)	I	Recurrent MG: MTD identified; 6% PR; 38% SD
PKC- β	Temsirolimus	I/II	Recurrent GBM: radiographic response: 5%–36%; PFS-6: 2.5%–7.8%
	Temsirolimus (+erlotinib)	I/II	Recurrent GBM: ongoing
	Everolimus (+AEE788)	I	Recurrent GBM: completed
	Enzastaurin	II	Recurrent GBM: 22% PR; 5% SD Recurrent AA: 24% PR; 13% SD
	Enzastaurin + carboplatin	I	Recurrent MG: ongoing
	Enzastaurin (+TMZ-RT)	I/II	Newly diagnosed GBM: ongoing
	Enzastaurin versus lomustine	III	Recurrent MG: discontinued because of lack of interim survival benefit
Multitargeted kinase inhibitors	Enzastaurin + bevacizumab	II	Recurrent MG: ongoing
	EGFR, VEGFR		
	AEE788	I	Recurrent GBM: completed
	Vandetanib (ZD6474)	I/II	Recurrent MG and progressive low-grade glioma: ongoing
EGFR, HER2/neu	Lapatinib	II	Recurrent GBM: ongoing
	Lapatinib (+pazopanib; VEGFR inhibitor)	I	Recurrent MG: ongoing
PDGFR, VEGFR	Sunitinib (SU11248)	I/II	Recurrent GBM: planned
	Sunitinib	II	Brain metastases in lung cancer: ongoing
FLT-3, PDGFR, c-KIT	Tandutinib (MLN518)	I/II	Recurrent GBM: ongoing
Miscellaneous			
Integrins	Cilengitide	I	Recurrent MG: MTD—not reached; 4% CR; 6% PR; 8% SD
	Cilengitide	II	Recurrent GBM: completed
	Cilengitide + TMZ/RT	II	Newly diagnosed GBM: PFS-6: 65%
Src	Dasatinib	II	Recurrent GBM: ongoing
HDAC	Vorinostat + TMZ	I	Malignant gliomas: ongoing
	Vorinostat	II	Recurrent GBM: completed accrual
	Depsiptide	I/II	Recurrent MG: ongoing

resistance. Glioblastoma stem cells promote tumor angiogenesis by secreting VEGF.¹²⁰ The effects of glioblastoma stem cells on angiogenesis can be specifically inhibited by bevacizumab. Brain tumor stem cells reside in a niche that may also represent a therapeutic target. Recent evidence suggests that modulation of some bone morphogenic proteins can decrease tumorigenic potential of glioma cancer stem cells.¹²¹ Also, targeting checkpoint kinases (CHK1 and CHK2) with small-molecule inhibitors can overcome the radioresistance of glioblastoma stem cells.¹²² Targeting cancer

stem cells may therefore represent a new therapeutic approach in glioblastomas.

Drug Delivery and Pharmacokinetics

Although most kinase inhibitors are small, they may not be able to cross the blood–brain barrier by their polarity; hydrophilicity; and active drug efflux transporters at the blood–brain, blood–cerebrospinal fluid, and blood–tumor barriers. Drug efflux transporters are also present on tumor cells, preventing intratumoral uptake of therapeutic agents.¹²³ Modulation of drug transporters may represent a

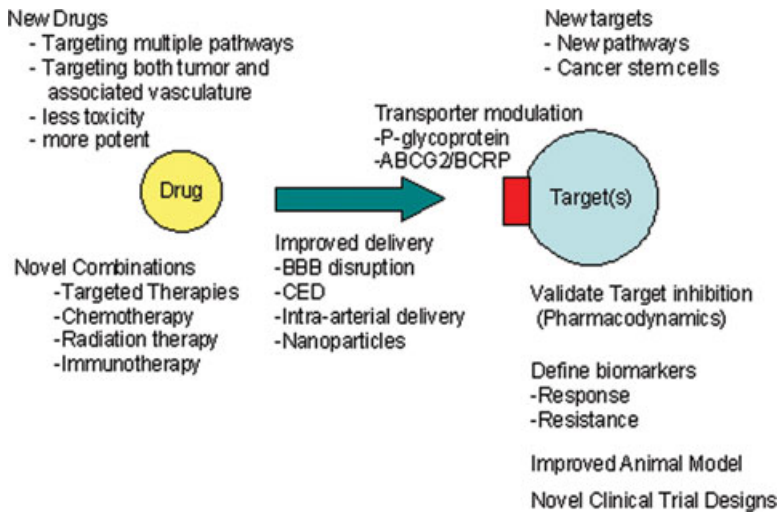


Figure 3. Strategies to improve effectiveness of designer drugs. ABCG2, ATP-binding cassette protein G2; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; CED, convection-enhanced delivery.

new potential strategy to improve efficacy of targeted agents.

CED is an approach to increase locoregional delivery of therapeutics that has been investigated in malignant astrocytoma.¹²⁴ Increased interstitial pressure in brain tumors may limit drug delivery from systemic vasculature and regular local infusion. CED uses the pressure gradient concept of continuous high-pressure, small-volume infusion over long periods (3–5 days) to optimize delivery of therapeutics in tumor/surgical bed via stereotactically placed catheters. Various therapeutic agents delivered by CED have been evaluated, including chemotherapies, gene/virus therapy, and ligand–toxin conjugates. Other new delivery approaches may include liposome-conjugated drugs, genetically modified stem cells, and nanoparticle delivery systems, which all are under preclinical development.

Pharmacokinetic evaluation is important in all phase I studies to determine drug level and potential drug–drug interactions. Also, some patients with brain tumors are treated with antiepileptic drugs. Several antiepileptic drugs such as phenytoin, phenobarbital, carbamazepine, oxcarbazepine, and primidone are hepatic cytochrome P450 inducers, which can

increase metabolism and decrease therapeutic levels of several targeted agents. Therefore, patients should be stratified into two groups in clinical trials on the basis of their coadministration of enzyme-inducing antiepileptic drugs (EIAEDs). Dosages between two arms should be escalated independently and pharmacokinetic studies should be performed. An alternative approach is to perform an initial phase I/II trial only in patients not on EIAEDs. If the new agent is found to be safe and efficacious, it may be further evaluated in patients on EIAEDs.

Multitargeted Inhibitors

First-generation kinase inhibitors, which disrupt only one or a few targets, have been associated with modest clinical benefit in unselected glioma patient populations. These failures may result from genetic heterogeneity and the existence of multiple parallel or compensatory pathways. Therefore, targeting only single kinases or pathways may not be sufficient for tumor control, unlike the success seen in chronic myelogenous leukemia and gastrointestinal stromal tumor treated with imatinib mesylate monotherapy.^{125,126} These two cancers exhibited “oncogene or pathway addiction”

(i.e., bcr-abl for chronic myelogenous leukemia and c-kit for gastrointestinal stromal tumor), which served as targets for imatinib mesylate.¹²⁷ Recent evidence has demonstrated concomitant activation of several receptor tyrosine kinases in glioma cell lines, xenografts, and primary glial tumors.¹²⁸ Simultaneous disruption of multiple kinases is more effective than inhibition of one kinase in decreasing downstream signaling, cell survival, and anchorage-independent growth. Currently, there are many multitargeted kinase inhibitors targeting multiple signal transduction pathways. AEE788 (Novartis) is a dual EGFR and VEGFR-2 inhibitor with *in vitro* and *in vivo* efficacy against glioblastoma.¹²⁹ A multicentered clinical study of AEE788 monotherapy in malignant gliomas has been completed. Vandetanib (ZD6474, Zactima; AstraZeneca), another inhibitor of EGFR/VEGFR-2, demonstrated cooperative effect with radiation and prolonged survival in murine models of intracranial glioma xenografts.¹³⁰ A phase I/II trial of vandetanib in malignant gliomas is ongoing. Sunitinib malate (Sutent, SU11248; Pfizer), an inhibitor of VEGFR-2, PDGFR, c-KIT, and FMS-like tyrosine kinase (FLT) 3, has antitumor activity against subcutaneous malignant glioma xenografts.¹³¹ A phase II study of sunitinib malate in malignant gliomas is ongoing. Tandutinib (MLN518, CT53518; Millennium Pharmaceuticals) is a new c-KIT and FLT-3 inhibitor that demonstrated efficacy in hematologic malignancies.¹³² A phase I/II trial of tandutinib in recurrent GBMs is ongoing. Clinical development of other multitargeted agents in malignant gliomas is in progress.

Combination and Multimodality Treatments

In addition to multitargeted inhibitors, combination therapy using agents that target different signaling pathways may circumvent tumor resistance to single-targeted inhibitors.¹³³ Strategy to determine the most promising combinations is important because the number of therapeutic combinations is almost limitless.¹³⁴

Currently, several strategies can be used to select targeted agents for combination therapy. Agents targeting the same pathway(s) may be combined to more potently block the activation of the pathway. For instance, a combination of cetuximab, a monoclonal antibody to EGFR, and gefitinib or erlotinib, an EGFR tyrosine kinase inhibitor, offers combinatorial antitumor benefit in a head and neck cancer model.¹³⁵ Clinical trials that disrupt two targets in the same pathway in malignant gliomas may include a phase I/II trial of sorafenib with erlotinib or tipifarnib. Cancer cells may compensate for the effects of specific molecular inhibitors (e.g., rapamycin) through the activation of feedback loops upstream from the primary target, suggesting that dual targeting upstream and downstream in a pathway may offer benefit. Clinical trials have been initiated based on this premise.

The second target can be a different but tumor-relevant cell type such as targeting endothelial cells in addition to tumor cells. A preclinical study demonstrated that combination of DC101 (VEGFR-2 antibody) and C225 (cetuximab [Erbix]) improved tumor control by inhibiting DC101-induced tumor cell migratory effect and vascular co-option.¹³⁶ Examples of clinical trials based on this concept may include a phase I trial of cetuximab, bevacizumab, and irinotecan; phase II trials of bevacizumab plus erlotinib or sorafenib or enzastaurin; and a phase I study of lapatinib plus pazopanib in recurrent malignant glioma.

The second target can be a parallel or compensatory pathway that may result in the resistance to the first agent. Because activation of the EGFR and/or PI3K pathway (by loss of PTEN) represents one of the most common genetic aberrations in GBM, several combination studies have focused on targeting EGFR and mTOR, downstream intracellular effectors in the PI3K pathway. Also, malignant gliomas that are resistant to EGFR inhibitors have demonstrated activation of the PI3K pathway through IGF-1R activation.⁷¹ Therefore, targeting both EGFR and PI3K pathways may

overcome the resistance and increase antitumor efficacy. A preclinical study of AEE788, a dual EGFR and VEGFR-2 inhibitor, and RAD001, an mTOR inhibitor, demonstrated *in vitro* and *in vivo* combinatorial benefits. Another study revealed greater antiproliferative and proapoptotic effects of EKI-785, an EGFR inhibitor, and rapamycin, than either agent alone in glioma cell lines.¹³⁷ Also, mTOR inhibition enhances sensitivity of GBM cells to EGFR kinase inhibitors, regardless of their PTEN status.¹³⁸ More recently, a combination of erlotinib and PI-103 (dual PI3K/mTOR inhibitor) has demonstrated superior efficacy in PTEN-mutant glioma to either monotherapy or therapy combining erlotinib with either PI3K inhibitor or mTOR inhibitor.¹³⁹ The concept of combining EGFR and mTOR inhibitors has translated into clinical trials such as erlotinib plus temsirolimus, gefitinib or erlotinib plus sirolimus, and AEE788 plus RAD001.^{140,141} A phase I trial of gefitinib plus sirolimus in recurrent malignant gliomas demonstrated safety and tolerability with encouraging antitumor activity.¹⁴⁰ In addition to EGFR inhibitors, an mTOR inhibitor, RAD001, has recently been combined with imatinib mesylate (PDGFR inhibitor) and hydroxyurea in a phase I trial for malignant glioma.

Preclinical studies have demonstrated combinatorial antiglioma benefit of combining VEGFR and PDGFR kinase inhibitors. A clinical study of imatinib mesylate/hydroxyurea and vatalanib in recurrent malignant glioma is ongoing.¹⁴² Furthermore, a clinical trial of imatinib mesylate/hydroxyurea in combination with vandetanib (EGFR/VEGFR inhibitor) in recurrent malignant glioma has recently been initiated.

Combinations of targeted agents that inhibit intracellular effectors in downstream parallel pathways have also been developed. Targeting the PI3K/AKT pathway through antisense oligonucleotides to integrin-linked kinase offered synergistic antitumor effects with small-molecule RAF-1 or MEK inhibitors.¹⁴³ Also,

a new RAF inhibitor, LBT613 (Novartis), and everolimus offer combinatorial benefits in blocking proliferation and invasion of glioma cell lines.¹⁴⁴ On the basis of this rationale, phase I/II trials of sorafenib and temsirolimus (mTOR inhibitor) are in progress. Several other promising combinations of targeted agents have undergone preclinical evaluation. A combination of sorafenib and a PKC- δ inhibitor, rottlerin, or a proteasome inhibitor, bortezomib, exhibited synergy in apoptosis induction of glioma cell lines.^{145,146} A combination of PI3K inhibitor, LY294002, and a chaperone protein heat shock protein 90 inhibitor, 17-AAG, demonstrated a combinatorial antiproliferative effect in glioma cell lines.¹⁴⁷ Clinical development of these combinations may be warranted.

Combinations of targeted agents with chemotherapies have been evaluated in malignant gliomas.¹⁴⁸ One of the most promising combinations is bevacizumab, an anti-VEGF antibody, plus irinotecan, a topoisomerase I inhibitor. Other chemotherapies currently under clinical investigation with bevacizumab include temozolomide and etoposide.

Radiation therapy has been a standard of care for malignant glioma. However, most patients eventually develop recurrence or progression after the treatment. Agents that can enhance or restore sensitivity of brain tumors to radiation therapy may improve patient outcome. Preclinical evidence demonstrated that radiation sensitivity may be regulated by growth factor signaling, DNA damage response protein activation, and apoptosis-related proteins. Among growth factor signaling pathways, EGFR was among the first that has been shown to contribute to radioresistance.¹⁴⁹ Clinical trials of gefitinib or erlotinib and radiation therapy (both conventional and stereotactic radiosurgery) are ongoing.¹⁵⁰ Other pathways such as PDGF, VEGF, and mTOR have also shown efficacy in enhancing radiation cytotoxicities through different mechanisms.¹⁵¹⁻¹⁵³ The sequence and timing of drug administration in relation to radiation therapy is crucial because

there is a significant difference in combinatorial effects in an animal study.¹⁵⁴

Identification of Biomarkers

Because only some patients who receive targeted agents have treatment benefit, identification of predictive biomarkers of response or resistance is a critical step to select the treatment for each cancer patient.¹⁵⁵ Also, biomarkers may serve as a pharmacodynamic measure to help monitor *in vivo* drug effect. In a recent phase I study of neoadjuvant rapamycin in patients with PTEN-deficient GBM, inhibition of mTOR activity as measured by reduced p70S6 kinase correlated with decreased tumor cell proliferation as measured by Ki-67 staining.¹⁵⁶ Biomarkers can help define the optimal biological dose for each targeted agent because the traditional maximal tolerated dose may not be the optimal dose for targeted agents to elicit antitumor effect.

Several recent studies using immunohistochemical analysis of archival tumor specimens have elucidated the molecular determinants for response to EGFR, VEGF, and mTOR inhibitors in malignant gliomas. These studies indicate technical feasibility of tumor immunohistochemistry for biomarker identification in malignant gliomas, which may serve as a paradigm of biomarker-guided targeted therapy if independently validated in larger prospective trials.

In addition to tissue biomarkers, other techniques may also serve as surrogates for response or resistance to therapy, possibly including circulatory markers and imaging biomarkers. Gene expression profiling has recently been used to predict response to chemotherapy or targeted agents in lung cancer.¹⁵⁷ This integrated genomic advance may serve as a foundation for personalized medicine for patients with cancers, including malignant glioma.

Improved Preclinical Models

Currently, there are several preclinical models for evaluating new therapeutic agents in

gliomas. Because malignant gliomas are genetically heterogeneous, even within one patient, using glioma cell lines with a restricted set of genetic abnormalities for drug evaluation may not be fully representative of actual human disease. Heterotopic and orthotopic xenograft systems are traditional models for preclinical testing of new drugs.¹⁵⁸ However, these models may fail to recapitulate the complex tumor microenvironment found in human tumors. Several new animal models have been developed to overcome this challenge. These models may include genetically engineered mouse models,¹⁵⁹ serially transplanted human xenograft models,¹⁶⁰ and cancer stem cell models.¹⁶¹ Each animal model has advantages and disadvantages, and some models face technical challenges. It remains to be elucidated which animal model serves best for screening of new therapeutics because no model has been systematically evaluated for predictive ability in clinical trials.

New Clinical Trial Designs

Because the number of targeted agents in development is rapidly increasing, selecting key candidates for clinical evaluation has recently become a challenge because of the limited number of glioma patients. New clinical trial designs have been developed to simultaneously evaluate several agents in a few patients in a timely fashion. Among these may include adaptive randomization and factorial designs. Adaptive randomization allows the simultaneous evaluation of several drugs in multiple treatment arms (testing each arm against the others). Interim outcome analysis is performed during accrual to adjust the randomization to enroll more patients into the arm with higher response rate. This design terminates ineffective agent(s) early in a trial while optimizing the number of patients in the most promising arm to achieve primary outcome analysis. Factorial design allows simultaneous evaluation of several therapeutic combinations with fixed and smaller accrual numbers of each arm.¹⁶²

Conclusion

Over the past 5 years, treatment for all cancers, including brain tumors, has shifted toward designer drug (targeted) therapy. First-generation targeted agents, which inhibit only one or a few kinases, have failed to demonstrate survival benefit as monotherapies in unselected patient populations. However, some patients harboring specific molecular abnormalities may have a favorable response to certain targeted agents. Identification of molecular/genetic profiles of tumors and correlative biomarkers of response or resistance to targeted therapies is therefore critical. Subsequently, each patient may be treated with an individualized therapeutic regimen based on molecular or genetic signatures. Meanwhile, several strategies have been developed to circumvent the poor response to current targeted agents. Such strategies may include new target identification, improved drug delivery, inhibition of multiple targets by multitargeted inhibitors or new treatment combinations, biomarker identification, reliable preclinical models, and new clinical trial designs and endpoints. Because the number of patients with primary brain tumors is limited, collaborative efforts among cancer centers both nationally and internationally will lead to expedited, efficient, and rational clinical trial evaluation of new therapeutic agents.

Conflicts of Interest

The authors declare no conflicts of interest.

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