Review

Lessons learned in the development of targeted therapy for malignant gliomas

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Abstract

The prognosis of patients with glioblastoma, anaplastic astrocytoma, and anaplastic oligodendroglioma remains poor despite standard treatment with radiotherapy and temozolomide. Molecular targeted therapy holds the promise of providing new, more effective treatment options with minimal toxicity. However, the development of targeted therapy for gliomas has been particularly challenging. The oncogenetic process in such tumors is driven by several signaling pathways that are differentially activated or silenced with both parallel and converging complex interactions. Therefore, it has been difficult to identify prevalent targets that act as key promoters of oncogenesis and that can be successfully addressed by novel agents. Several drugs have been tested, including epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (gefitinib and erlotinib), mammalian target of rapamycin (mTOR) inhibitors (temsirolimus and everolimus), and vascular endothelial growth factor receptor (VEGFR), protein kinase C-β, and other angiogenesis pathways inhibitors (vatalanib, bevacizumab, and enzastaurin). Although preliminary efficacy results of most trials in recurrent disease have fallen short on expectations, substantial advances have been achieved by associated translational research. In this article, we seek to recapitulate the lessons learned in the development of targeted therapy for gliomas, including challenges and pitfalls in the interpretation of preclinical data, specific issues in glioma trial design, insights provided by translational research, changes in paradigms, and future perspectives. [Mol Cancer Ther 2007;6(7):1909–19]

Introduction

Malignant gliomas are the most common type of primary brain tumors affecting ~16,000 new patients every year in the United States. The term malignant glioma comprises WHO grade IV tumors, such as glioblastomas with its variants, and WHO grade III tumors (anaplastic forms of astrocytoma, oligodendroglioma, and oligoastrocytoma). Glioblastoma is by far the most frequent malignant glioma and is associated with a particularly aggressive course and dismal prognosis. Despite numerous clinical trials, little improvement in overall survival (OS) or progression-free survival (PFS) has been achieved in the past 20 years. Surgical resection and radiotherapy have been the mainstay of treatment; only recently have the benefits of chemotherapy been unequivocally shown in a randomized trial (1). In that study, the combination of temozolomide with radiotherapy followed by adjuvant temozolomide was tested against radiotherapy alone in patients with newly diagnosed glioblastoma. The chemotherapy arm achieved improved OS (median, 15 months versus 12 months; 2-year OS, 27% versus 10%) and PFS (median, 7 months versus 5 months). Despite the clear benefit, such improvements remain modest from a clinical standpoint. Outcomes in recurrent or progressive glioblastoma are even less favorable (median OS, 6–7 months; median PFS, 2–3 months; refs. 2, 3), reflecting the minimal benefits of salvage treatment. WHO grade III tumors have a relatively better prognosis, with a median OS of 2 to 4 years for newly diagnosed anaplastic astrocytomas and 3 to 5 years for anaplastic oligodendrogliomas, which are chemosensitive tumors (4–6). Yet, even for those, recurrence is the norm, and disease will follow a fatal course in virtually all patients with malignant glioma. New treatment alternatives, such as molecular targeted therapy, are thus clearly needed.

Challenges and Pitfalls in the Development of Targeted Therapy for Malignant Gliomas

Preclinical Data: The Complexity of Glioma Cell Biology

A first step in the process of developing targeted therapy is the identification of highly prevalent targets that constitute key master promoters of oncogenesis in specific tumors. Malignant gliomas are highly heterogeneous tumors from a molecular stand point; several signaling pathways have been found to be differentially activated or silenced, with both parallel and converging

complex interactions. Examples of such pathways include growth factors, phosphatidylinositol 3-kinase (PI3K)/ AKT/PTEN/mammalian target of rapamycin (mTOR), Ras/Raf/mitogen-activated protein kinase (MAPK) kinase (MEK)/MAPK, and sonic hedgehog/PTCH (7–9). Clues for the identification of key potential targets in the midst of such complexity are provided by the establishment of associations between a given molecular abnormality and its prognosis. However, in gliomas, the large heterogeneity and low prevalence of each molecular abnormality have decreased the statistical power of studies seeking to establish their prognostic implications. A majority of such studies either have found no significant associations or have reported conflicting results (10–12). Misinterpretation of such studies might lead to overestimation of the biological relevance of a prevalent molecular abnormality or nonrecognition of low-prevalence but high-relevance targets. Thus, establishment of optimal targets in gliomas

is a process that needs to be continuously reassessed and validated by additional laboratory and clinical trial data.

Once a potential target is identified, the natural next step involves discovering new agents capable of restoring normal cell functions through interaction with the target. Currently, array-based screening of drug compound libraries has become the norm; several new molecules have been identified and are in different stages of development (Fig. 1). However, preclinical evaluation of new compounds in malignant gliomas has encountered additional challenges. The value of existing *in vitro* and animal model studies in predicting drug efficacy in human gliomas has been increasingly questioned (13). Available glioma cell lines for *in vitro* assays do not recapitulate the whole spectrum of molecular abnormalities that are typically found in a population of human tumors. Optimal genetically modified animal tumor models have not been validated, and heterotopic or orthotopic xenografts may
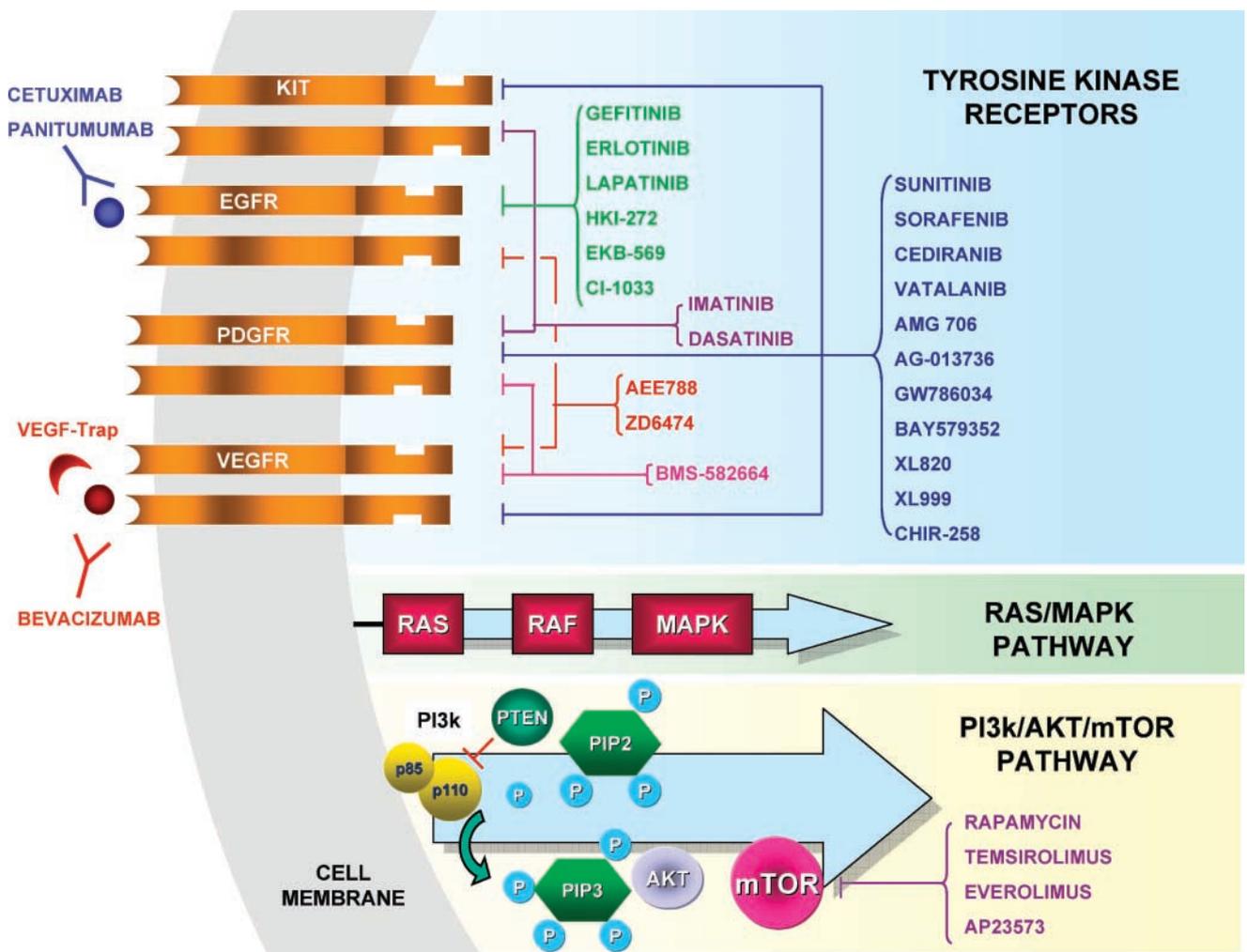

Figure 1. Schematic representation of main oncogenetic signaling molecular pathways and corresponding single and multitargeted drugs under development.

not reconstitute the complexity of microenvironment interactions, invasiveness, and angiogenesis found in such tumors. Therefore, separating promising from nonpromising drugs based on preclinical evidence remains difficult.

Clinical Trials in Gliomas: Methodologic and Ethical Issues

Additional challenges are encountered in the clinical stages of drug development. Brain tumor patients are typically excluded from phase I trials, allegedly because neurologic deficits may compromise their performance status and preclude adequate evaluation of toxicities. Extrapolating phase I nonbrain tumor results to gliomas is difficult due to several characteristics unique to this type of tumor. Many phase I trials do not adequately address pharmacokinetic interactions with enzyme-inducing drugs, such as anticonvulsants, which are frequently used in glioma patients. Moreover, such trials frequently do not include assessment of the ability of the agent to cross an intact blood-brain barrier. Blood-brain barrier crossing properties may be a relevant variable in drug development for gliomas because, although the blood-brain barrier may be disrupted in some areas of the tumor, a substantial proportion of glioma cells are located under areas with an intact blood-brain barrier, thus permeating normal brain. Recent shifts in phase I paradigms may further complicate this issue. Many phase I studies are seeking to establish an ''optimal biological dose'' rather than the traditional ''maximum tolerated dose'' through the use of surrogate markers of activity. However, such studies have not checked whether this optimal biological dose is also being reached within the central nervous system, which would be important in the treatment of gliomas (14). Another issue in the extrapolation of phase I data from solid tumors to brain tumors is the possibility of intratumoral bleeding and necrosis that is occasionally seen with the use of targeted agents with antiangiogenic properties (15). Although such complications are typically manageable in other types of tumors and may not prevent dose escalation, they can be extremely disabling and life threatening in intracranial tumors, such as gliomas. For all these reasons, phase I trials usually have to be redone in malignant gliomas, thus further slowing the process of drug testing.

Other challenges are faced once candidate drugs reach phase II trials. Optimal designs and end points for targeted therapy have been intensively debated. Strict response criteria based on magnetic resonance imaging/computed tomography contrast enhancement have been proposed for evaluation of response in malignant gliomas in an effort to account for differences in imaging acquisition techniques and the effects of corticotherapy on enhancing disease (16). However, some have questioned the validity of such strict criteria as a measure of efficacy of therapies that seem to be associated with a cytostatic rather than a cytotoxic effect (17). This issue also has major implications for the statistical analysis of correlative studies seeking to establish surrogate markers of clinical benefit. As an example, it remains unknown whether the large cohort of patients with stable disease should be considered as having benefited from a treatment or not. Alternative phase II trial designs, surrogate markers, radiographic criteria, and statistical methods have been proposed to surpass these difficulties, but their validity remains to be shown. For the time being, PFS (typically 6-month PFS) remains the most frequently recommended primary end point for glioma trials because it is assumed to be a surrogate marker of preservation of neurologic and functional status as well as a predictor of OS. However, it is of note that such assumptions lack objective validation and cannot be universally applied. Because assessment of PFS depends on radiographic evaluation, once again it is possible that mismatches between biological activity and radiographic effects as seen in certain types of treatment (i.e., local therapies and antiangiogenic drugs) may limit the value of PFS as a predictor of clinical benefit or survival. Thus, the choice of primary end points should still be individualized according to type of treatment.

A final challenge in drug development for malignant glioma is posed by difficulties in the process of obtaining tissue for correlative studies. Such studies are essential to identify molecular markers of response and to better understand the mechanisms of action of a drug using the so-called ''bench-to-bedside-to-bench'' approach. Obtaining tissue from brain tumor patients requires neurosurgical procedures, the risks of which constitute an ethical challenge. Frequently, malignant gliomas are inoperable and only stereotatic biopsies that provide minimal amounts of tissue are done. Gliomas frequently exhibit intratumoral heterogeneity in terms of molecular expression; thus, small samples may not represent the entirety of the tumor (18). Moreover, new drugs are frequently tested in recurrent disease, from which tissue is not available. Recurrent tumor may be different from the newly diagnosed tumor in terms of genetic expression and relevance of specific targets; thus, correlative studies may not capture meaningful associations. Attempts have been made to incorporate ''surgical arms'' into clinical trials for recurrent gliomas in which only patients who are candidates for surgery for medical reasons are allowed (19). Such a strategy constitutes an ethically acceptable way of doing relevant tissue analyses *in vivo*. Unfortunately, accrual is typically slow because of the low number of patients with a clear medical indication for surgery.

Despite all the challenges, intense research is ongoing and substantial progress has been made toward identifying and overcoming existing limitations. In the following discussion, we seek to recapitulate the lessons learned in the process of testing targeted therapies for gliomas, with a review of the rationale for most of the commonly proposed targets, results of first clinical trials, associated translational research, and future perspectives.

Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR; ErbB1, HER1) is a tyrosine kinase receptor that is abnormally activated in 70% of solid cancers. Activation of EGFR pathways in

cancer cells has been linked to increased motility, adhesion, invasion, and proliferation of tumor cells as well as inhibition of apoptosis and induction of angiogenesis. The EGFR transmembrane protein comprises three domains: an extracellular ligand-binding domain, a transmembrane lipophilic region, and an intracellular tyrosine kinase domain. Ligands, such as EGF and transforming growth factor-α, bind to the extracellular domain of two receptors simultaneously, resulting in two receptors bound together at the cell surface (receptor dimerization). This leads to modification of the three-dimensional structure of the receptor and induces intracellular cross-phosphorylation between the tyrosine kinase subunit of one receptor and the kinase of the other receptor. Such activation processes promote signal transduction and gene activation mediated by several downstream signaling pathways, including the PI3K/AKT/mTOR, Ras/Raf/MAPK, and protein kinase C signaling pathways.

EGFR is amplified or overexpressed in up to 60% of glioblastomas (20, 21). Such alterations are a hallmark of the so-called ''primary'' glioblastoma as opposed to glioblastomas secondarily arising from lower-grade gliomas in which such abnormalities are rare. Mutations of the *EGFR* gene are frequent in glioblastomas and may be present in up to 50% to 70% of EGFR-overexpressing tumors. Most mutations affect the extracellular domain and involve a large deletion in exons 2 to 7. The resulting variant receptor, termed EGFRvIII, has ligand-independent kinase activity and is observed in 60% to 70% of EGFR-overexpressing glioblastomas (21). Several studies have tried to link EGFR expression patterns and differential prognosis in glioblastomas, but conflicting results have been found. This seems to be explained by use of varying methodologies, including different assessment techniques of EGFR expres- sion, heterogeneous patient characteristics, and often small sample sizes. Some studies have suggested that EGFRvIII is associated with a less favorable prognosis (22, 23), whereas such association is less clear in wild-type EGFR-over- expressing tumors (10–12).

Several strategies targeting EGFR in glioblastomas have been proposed, including the use of monoclonal antibodies against EGFR or EGFRvIII, bispecific antibodies, toxin- linked conjugates, vaccine therapies, and small-molecule tyrosine kinase inhibitors (EGFR TKI; ref. 21). Most current research efforts are concentrated on EGFR TKIs, which are in more advanced stages of development in other tumors, such as lung cancer. These drugs act by competing with ATP for binding to the kinase pocket of the receptor, thus blocking receptor activation and transduction of postre- ceptor signals. Results of the first EGFR TKI phase II trials, such as gefitinib (ZD-1839) and erlotinib (OSI-774) in recurrent and newly diagnosed glioblastoma (Table 1) and associated correlative studies (Table 2) are becoming available (24–30). Phase I data showed that the drugs were well tolerated, although dose escalations were necessary in patients on enzyme-inducing drugs (31). However, phase II data (Table 1) suggested that, although some responses were obtained, the overall efficacy of such compounds in unselected patients was minimal when compared with historical data (2, 3). Interpretation of correlative studies linked to these trials (Table 2) have been limited by the low number of responders and by the different techniques used (24, 30, 32, 33). Such studies confirmed previous reports suggesting that the EGFR tyrosine kinase domain mutations that predict response to EGFR TKI in lung cancer are absent in gliomas (34, 35), including in those that allegedly responded to EGFR TKI. Most studies found no correlation between outcomes and

Table 1. Small-molecule EGFR TKIs: phase II trials in recurrent and newly diagnosed glioblastoma and comparison with historical controls

Reference	Dose	*n*	Objective response (CR + PR), %	Disease control (CR + PR + SD), %	Median PFS (mo)	6-mo PFS (%)	Median OS
Gefitinib (recurrent GBM)							
Rich et al. (24)	500–1,000 g	53	0	42	2	13	10
Lieberman et al. (25)*	500–1,500 g	38	13 (5 PR)	NA	2	9	NA
Franceschi et al. (26)*	250 mg	16	0	13	2	12	6
Gefitinib (newly diagnosed GBM)							
Uhm et al. (27)*	500–1,000 g	98	NA	NA	7	NA	11
Gefitinib + everolimus (recurrent GBM)							
Nguyen et al. (50)*	250 mg (gefitinib); 30–70 mg (everolimus)	19	10 (2 PR)	47 (including 2 minor responses)	3	NA	7
Erlotinib (recurrent GBM)							
Raizer et al. (28)*	150 mg	30	0	13	3	0	NA
Cloughesy et al. (29)*	150–500 mg	48	8 (1 CR; 3 PR)	41	2	17	NA
Vogelbaum et al. (30)*	150 mg	31	26 (8 PR)	42	3	26	6
Historical controls (recurrent GBM; refs. 2, 3)			5–6	33–42	2–3	15–21	6–7

Abbreviations: GBM, glioblastoma; CR, complete response; PR, partial response; SD, stable disease; NA, not available.
*Preliminary results presented in the form of abstract.

EGFR overexpression or amplification (19, 24, 30, 33), with the exception of one study that found that tumors with high levels of EGFR expression and low levels of phosphorylated AKT/protein kinase B (pAKT) were more likely to respond than those with low levels of EGFR expression and high levels of pAKT (32). Whereas smaller studies found no correlation between the presence of EGFRvIII and outcomes (32, 36), a larger study validated in an independent data set found that coexpression of EGFRvIII and PTEN was statistically significantly associated with response (33), which partially contradicts preclinical data suggesting that EGFRvIII-expressing cell lines are resistant to gefitinib (22, 37). Another recent study reported the first results of surgical arms evaluating the molecular effects of EGFR TKI *in vivo* (19). Comparative analysis of tissue obtained from patients before and after the start of treatment suggested that EGFR phosphorylation and downstream signaling were not markedly inhibited after treatment was started. However, interpretation of findings was limited by several technical aspects. Among the patients defined as ''sensitive to treatment,'' only one patient had a sustained objective radiographic response and posttreatment tissue for that patient was not available; all other ''sensitive tumors'' had achieved only a short-lived stable status. Moreover, no patient expressed EGFRvIII, which illustrates how small sample sizes may be biased with regard to the prevalence of single molecular abnormalities. Finally, pharmacokinetic analysis of tissue penetration found conflicting results, with some tumors showing minimal intratumoral drug concentrations whereas others showed higher concentrations, raising the possibility of contamination by blood clots. Despite the lack of definitive conclusions, that study has major merit in showing the feasibility and potential usefulness of surgical arms and highlights the importance of interinstitutional sharing of data and tissue.

As discussed above, translational research studies linked to EGFR TKI have found somehow conflicting findings and have not provided definitive explanations on why such drugs have not been effective. In spite of this, a few general conclusions are possible. It became clear that the mechanisms of sensitivity and resistance in gliomas differ from other types of tumors, such as lung cancer, in that the sensitivity in gliomas does not seem to be linked to tyrosine kinase domain mutations. Moreover, it seems that isolated EGFR expression as assessed by traditional methods does not seem to predict response to EGFR TKI, although all results seem to point to the EGFR/PI3K/mTOR pathway components as potential markers of activity. Ongoing trials of gefitinib, erlotinib, and other EGFR TKIs, such as lapatinib, as well as further tissue analysis of finished trials, may clarify whether selection of patients based on EGFR overexpression, EGFRvIII, pAKT, and/or PTEN expression could improve results. Such studies should also clarify whether poor tumor penetration plays a role in the lack of efficacy of such drugs; further evaluation of downstream signaling should provide insight on whether such drugs are really hitting the target. Other trials combining EGFR TKI with alternative targeted agents may answer whether acting on downstream or collateral pathways could result in synergistic effects (further discussed below). Other attempts to overcome resistance include trials combining EGFR TKI with cytotoxic chemotherapy and radiotherapy as well as the use of new agents that are capable of inhibiting EGFRvIII-overexpressing cell lines *in vitro*, such as HKI-272 (an irreversible EGFR TKI) and AEE788 [a multitarget ErB2/vascular endothelial growth factor receptor (VEGFR)/EGFR TKI; refs. 38, 39].

Mammalian Target of Rapamycin

Overactivation of the PI3K/AKT/mTOR pathway seems to play a key role in the downstream signaling pathways promoting growth and survival in several types of tumor cells, including glioma (8, 40). Activation of such a pathway is triggered by stimulation of growth factor receptors [including EGFR, platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor, and insulin-like growth factor-I receptor] and the Ras pathway, leading to activation of PI3K. Activated PI3K transforms PIP2 in PIP3 through a process regulated by the tumor suppressor gene *PTEN*. Activated PIP3 promotes phosphorylation of AKT through translocation near the cell membrane and activation of PDK1 and PDK2. pAKT promotes phosphorylation of several downstream effectors, including MDM2, p21/p27, Bad, FKHR, nuclear factor-κB, caspase-9, glycogen synthase kinase-3β, and mTOR. mTOR plays a key role in downstream signaling of the PI3K/AKT pathway through the regulation of cellular catabolism, anabolism, proliferation, cell cycle control, autophagy, angiogenesis, and apoptosis. Major mTOR downstream pathways include the p70s6k and 4E-BP1 pathways. *In vitro* studies have suggested that mTOR activity is particularly highly activated in cells with deficient PTEN function, including glioma cell lines (8). Alterations of PTEN expression are frequent in high-grade gliomas (up to 65% of glioblastomas), with *PTEN* mutations being present in 15% to 40% of primary glioblastomas (41, 42). These alterations in PTEN expression seem to be associated with a worse prognosis, although, again, conflicting results have been reported (20, 42–44). Preclinical studies in gliomas have suggested that PTEN-deficient tumors show enhanced sensitivity to mTOR inhibition; this provided the rationale for clinical trials of mTOR inhibitors in glioblastomas (45, 46). Phase II results of two studies using temsirolimus (CCI-779) are available (Table 3; refs. 17, 47). Both studies found that, although drug metabolism was significantly affected by enzyme-inducing anticonvulsants, therapeutic serum levels could still be achieved with a dose of 250 mg/wk. However, in terms of efficacy, results in unselected patients were disappointing with no improvement in response rates, PFS, or OS. The paucity of responses prevented adequate correlative studies in the search of markers of response. Nonetheless, correlative analyses in one of the studies were done using unconventional response criteria, defining ''tumor regression'' as any unequivocal reduction

Table 2. Correlative studies linked to gefitinib and erlotinib clinical trials

Reference	EGFR wild-type immunohistochemistry	EGFR amplification	EGFRvIII
Rich et al. (gefitinib; ref. 24)	No correlation with TTP	No correlation with TTP	No correlation with TTP
Vogelbaum et al. (erlotinib; ref. 30)*	—	No correlation with ORR	—
Haas-Kogan et al. (erlotinib; ref. 32)	Response in 3/7 overexpressing tumors and 0/10 nonoverexpressing ($P = 0.03$)	Response in 3/6 amplified tumors and 2/22 nonamplified ($P = 0.02$)	No correlation with ORR
Mellinghoff et al. (gefitinib/erlotinib; ref. 33)	No correlation	No correlation	Response in 6/12 tumors with EGFRvIII and 1/14 without EGFRvIII ($P = 0.03$)
Lassman et al. (gefitinib/erlotinib— surgical arms; ref. 19)	—	No correlation with ORR	—

Abbreviations: PKB, protein kinase B; TTP, time to progression; ORR, objective response rates.
*Preliminary results presented in the form of abstract.

in the size of contrast enhancement, decrease in the mass effect in patients, or decrease in the stable steroids dose needed. Grade 2 or higher hyperlipidemia as well as expression of p70s6k were predictors of response (both $P = 0.04$), whereas EGFR amplification, PTEN deletion (fluorescence *in situ* hybridization), PTEN expression (immunohistochemistry), AKT, and pAKT were not (17). Taken together, these studies suggest that temsirolimus has limited activity in glioblastomas as a single agent; no obvious subpopulation of patients that could benefit from treatment has been reliably identified. Prompted by *in vitro* evidence of synergism between mTOR inhibitors and EGFR TKI (48), ongoing clinical research is concentrating efforts on such combinations, including trials of temsirolimus, everolimus (RAD001), or sirolimus (rapamycin) combined with gefitinib, erlotinib, or AEE788. A phase I study

Table 3. Results of various targeted therapy phase II trials in recurrent glioblastoma

Reference	Dose	n	ORR (CR + PR), %	Disease control (CR + PR + SD), %	Median PFS (mo)	6-month PFS (%)	Median OS (mo)
Temsirolimus							
Chang et al. (47)	170–250 mg weekly	43	5	47	2.3	3	
Galanis et al. (17)	250 mg weekly	65	0	NA	2.3	8	4
Imatinib							
Raymond et al. (15)*	600–800 mg	51	4	35	2.3	16	NA
Wen et al. (56)	600–800 mg	34	6	24	NA	3	NA
Imatinib + hydroxyurea							
Dresemann (57)		30	20	57	2.5	32	4.8
Reardon et al. (58)	Imatinib, 400–1,000 g; hydroxyurea, 500 mg	30	9	—	3.6	27	12
Vatalanib							
Conrad et al. (phase I/II; ref. 63)*	150–2,000 mg	47	4	56	NA	NA	NA
Vatalanib + TMZ or CCNU							
Reardon et al. (phase I/II; ref. 64)*	500–1,500 mg (TMZ, 200 mg/m²; CCNU, 130 mg/m²)	51	8	53	4 (TMZ); 2.5 (CCNU)	NA	NA
Bevacizumab + irinotecan							
Vredenburgh et al. (74)	10 mg/kg (irinotecan, 125 mg/m²)	23	61	96	5	30	10
Enzastaurin							
Fine et al. (78)*	500–900 mg	63	22	27	NA	NA	NA

Abbreviations: TMZ, temozolomide; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea.
*Preliminary results presented in the form of abstract.

Table 2. Correlative studies linked to gefitinib and erlotinib clinical trials (Cont'd)

EGFR tyrosine kinase mutation	EGFR activity phosphorylated EGFR	PTEN mutation/loss	PKB/AKT
—	—	—	—
—	—	—	—
—	—	No correlation with ORR	Response 0/18 in activated AKT and 5/11 in nonactivated ($P = 0.02$)
None found	—	Response in 7/13 without PTEN loss and 0/13 with PTEN loss ($P = 0.005$)	—
None found	No decrease after treatment	—	pAKT decreased in 2 responders

combining gefitinib and sirolimus in malignant gliomas found no significant pharmacokinetic interaction between the two drugs, but pharmacokinetics of both drugs were significantly affected by enzyme-inducing drugs. Of note, a partial response was seen in 2 of 34 patients and disease remained stable in 13 of 34 patients. No obvious molecular markers of response could be defined (49). Preliminary results of a study using gefitinib and everolimus in unselected recurrent glioblastoma patients were recently presented. Using modified radiographic criteria, responses were found in 31% of patients, including partial and minor responses. However, median OS and PFS were not different from historical controls (50). Results of trials of similar combination of drugs but enrolling only selected patients with evidence of PI3K/AKT/mTOR activation are awaited.

Platelet-Derived Growth Factor Receptor

PDGFs are a growth factor family composed of four different polypeptide chains (PDGF-A, PDGF-B, PDGF-C, and PDGF-D) that exert their cellular effects through two types of protein tyrosine kinase receptors: PDGFR-α and PDGFR-β (51). Ligand binding induces receptor dimerization, activation, and autophosphorylation of the tyrosine kinase domain, which results in activation of several signal transduction pathways, including Ras-MAPK, PI3K, Src family kinase, signal transducers and activators of transcription factors (Stat), and phospholipase Cγ. Overexpression of PDGF and PDGFR has been shown to play a role in the development of cancer through autocrine stimulation of cancer cells, development of angiogenesis, and control of tumor interstitial pressure. PDGFR and PDGF are frequently expressed in gliomas, particularly in secondary glioblastomas (52). Such overexpression seems to result mainly from deregulated expression and is associated with TP53 tumor suppressor gene loss. A few direct genetic abnormalities have also been identified, such as PDGFR-α amplification and a constitutive activating

mutation consisting of a deletion in exons 8 and 9. *In situ* hybridization has shown that PDGFR-α and PDGF are expressed mainly in tumor cells, whereas endothelial cells express PDGFR-β; this suggests that paracrine effects are also relevant (51, 52). PDGF and PDGFR expression seems to be associated with a poorer prognosis in gliomas. Animal gliomagenesis models have suggested that PDGFR pathways not only play a role in proliferation but also have effects on cell differentiation through dedifferentiation of mature cells, prevention of glial cell differentiation, and even promotion of cancer stem cells (53, 54). A role for PDGFR as a potential target for treatment of gliomas was suggested by the inhibition of growth in cell lines and xenograft models induced by PDGFR TKIs (55). Inhibition of PDGFR was correlated with decreased phosphorylated extracellular signal-regulated kinase and pAKT levels, suggesting inhibition of MAPK and PI3K pathways. Several inhibitors of PDGFR have been developed; most of them are not specific for PDGFR and act on other tyrosine kinases as well (51). Imatinib has been the most studied drug of this class. Imatinib blocks the activity of the Bcl-Abl, c-kit, and PDGFR and has been approved for chronic myeloid leukemia and gastrointestinal stromal tumors. Phase I data in malignant gliomas have suggested that use of enzyme-inducing drugs significantly affects drug metabolization (56). Despite the increased rates of intratumoral hemorrhages, the drug has been well tolerated overall. However, preliminary phase II results of single-agent imatinib have found only limited efficacy in unselected patients (Table 3; refs. 15, 56). One of the studies found that results were especially disappointing (6-month PFS of 10%) in grade III gliomas in which PDGFR pathways are thought to play an important role (56). Because of the low response rates in both studies, attempts of establishing molecular markers of efficacy and evaluating downstream effects have failed. Therefore, it remains unknown whether the lack of efficacy is explained by poor tumor penetration or by a lack of a key role of PDGF pathways in such tumors. Conversely,

two studies using a combination of imatinib and hydroxyurea achieved better results compared with imatinib alone or historical controls (Table 3; 57, 58). It has been hypothesized that imatinib potentiates hydroxyurea cytotoxic effects through a decrease in tumor interstitial pressure (which would increase hydroxyurea delivery to tumor cells) and a decrease in DNA repair secondary to imatinib-induced reduction of Rad 51 expression. However, because of small sample sizes and possible selection bias, results of such trials have been interpreted cautiously, with confirming trials currently under way. Ongoing trials of the combination of imatinib with other cytotoxic agents may provide further insight on the mechanisms of such synergism, if real.

Vascular Endothelial Growth Factor Receptor

Tumor angiogenesis is a complex process that involves several steps, including the breakdown of basement membrane, endothelial cell proliferation, cell to cell and cell to matrix interactions, and mobilization of circulating endothelial cells and hematopoietic progenitors (59). Each of these steps involves several molecular alterations that can be potentially targeted therapeutically. VEGF-A is a member of the VEGF family that acts as a key proangiogenic factor because of its specificity to endothelial cells and the multitude of responses that it can elicit. These include extracellular matrix degradation, endothelial cell proliferation, migration and tube formation, and expression of other proangiogenic factors, such as urokinase-type plasminogen activator, plasminogen activator inhibitor-1, urokinase-type plasminogen activator receptor, and matrix metalloproteinase-1. Overexpression of VEGF-A occurs in response to hypoxia (through hypoxia-inducible factor-1), PDGF, EGF, transforming growth factor-β, interleukin-1β, and tumor necrosis factor-α. The main receptors involved in relaying VEGF-A signaling are VEGFR-1 (Flt-1) and VEGFR-2 (KDR, Flk-1). The best-studied receptor is VEGFR-2, a potent tyrosine kinase that mediates endothelial cell signaling through the activation of Ras/Raf/MEK/MAPK, PI3K/AKT/PKB, and protein kinase C-β pathways. Gliomas are highly vascularized tumors and have been shown to overexpress VEGF-A; such expression has been linked to a poorer prognosis (60, 61). Other studies suggest that blocking VEGF pathways may normalize tumor vasculature and improve chemotherapy delivery, allowing higher drug concentrations (59). Several strategies for targeting VEGF have been proposed, such as anti-VEGF-A and VEGFR-2 monoclonal antibodies, antisense oligonucleotides, ribozymes, and VEGFR TKI. Most VEGFR TKI drugs are not specific and act on other tyrosine kinase receptors. The best-studied drugs in gliomas have been vatalanib (PTK787/ZK222584), ZD6474, sorafenib (BAY 43-9006), sunitinib (SU11248), and cediranib (AZD2171). Testing such antiangiogenic drugs has been particularly challenging because of the lack of well-established paradigms for clinical trial design. Difficulties include defining the

optimal biological dose of drugs that are rarely toxic, determining the relevance of combinations with cytotoxic chemotherapy and radiotherapy, and elucidating adequate surrogate markers.

Vatalanib is a VEGFR-1 and VEGFR-2 TKI that showed activity in glioma cell lines and xenograft models (62). Preliminary results of two phase I/II studies in recurrent glioblastoma have been presented (Table 3; refs. 63, 64). In one study, among the 47 evaluable patients treated with single-agent vatalanib, 4% achieved a partial response and 56% achieved stability in their disease (for a median duration of stabilization of 3 months). Evaluation of surrogate markers, such as tumor blood supply, by dynamic contrast-enhanced and dynamic susceptibility change magnetic resonance imaging showed dose-dependent decreases in vascular permeability and cerebral volume (63). In another phase I/II study, vatalanib was tested in combination with either temozolomide or lomustine in recurrent glioblastoma. The combination with temozolomide seemed better tolerated, but maximum tolerated dose had not been reached. Among 51 evaluable patients, 8% had a partial response and 53% were stable; the median time to progression was 16 weeks for patients in the temozolomide group and 10 weeks for patients in the lomustine group (64). These promising preliminary results, particularly in combination with cytotoxic chemotherapy, have prompted other studies in the newly diagnosed setting using vatalanib in combination with temozolomide.

ZD6474 is a VEGFR-2 TKI with additional VEGR-3 and EGFR inhibition properties. *In vitro*, such compound has a spectrum of action similar to gefitinib, highlighting its anti-EGFR activity; EGFR tyrosine kinase–mutated lung cells seem particularly hypersensitive to this drug. *In vivo* models showed a broader spectrum of action, suggesting an antiangiogenic and anti-VEGFR preponderant effect (65). Phase II trials have shown modest activity in lung cancer and disappointing results in other solid tumors, raising doubts on whether EGFR inhibition, rather than VEGF inhibition, was responsible for the shown activity (66). In a glioma xenograft study, ZD6474 decreased tumor volume, lowered the tumor cell proliferation index (Ki-67), and increased tumor cell apoptosis. However, microvascular density, a typical marker of angiogenic activity, surprisingly increased, raising doubts on the antiangiogenic effects of the drug (67). Another study in various brain tumor xenografts found that some tumors that were insensitive to TKI specifically targeting EGFR or VEGFR alone were sensitive to ZD6474, suggesting that combined EGFR and VEGFR targeting may significantly increase tumor control (68). Other xenograft studies have shown that the combination of ZD6474 with temozolomide (69) and radiotherapy (70) is synergistic, providing rationale for ongoing clinical trials.

Cediranib (AZD2171) is an oral pan-VEGFR TKI that is also being currently tested in malignant gliomas; results of translational research associated to an ongoing phase II

trial are available (71). In that study, drug effects were evaluated over time through the use of magnetic resonance imaging techniques (including analysis of perfusion, permeability, and relative vessel size) and assessment of circulating progenitor cells, circulating endothelial cells, and plasma levels of several proangiogenic proteins. Results suggest that cediranib leads to a normalization of vasculature and decreased edema; however, such effects disappear overtime, which is accompanied by an increase in circulating basic fibroblast growth factor, stromal cell–derived factor-1α, and circulating endothelial cells. Despite the small sample size (n = 16), such study has the merit of showing the feasibility and relevance of the studied surrogate markers and provides some insight on the mechanisms of the short-lived nature of antiangiogenic responses in recurrent glioblastoma, while final efficacy results are awaited.

Preclinical evidence of activity in gliomas is also available for sunitinib, a multitargeted VEGFR-2 and PDGFR inhibitor (72, 73). Of note, a study using sunitinib in xenograft models observed development of tumor necrosis and hemorrhage in 20% of animals, suggesting that careful dose escalation would be necessary in humans. It remains unknown whether such ability in inducing necrosis and hemorrhage could be a marker of increased antiangiogenic (and thus antitumor) activity; planned clinical trials will investigate such hypothesis.

More recently, a single-institution phase II trial using the humanized monoclonal VEGF antibody bevacizumab combined with irinotecan in malignant gliomas has been reported (74). No central nervous system hemorrhages were observed, although it is of note that strict safety criteria were used (concomitant anticoagulation was an exclusion criteria, and patients requiring anticoagulation while on treatment were taken off study). As thromboembolic complications were frequent, high dropout rates were observed, with a total of 12 patients taken off study in the absence of disease progression. Regardless, impressive response rates were observed, with 63% of the 32 enrolled patients showing at least a partial response (Table 2). Improvements in PFS and OS were less impressive (glioblastoma patients: median PFS, 20 weeks; median OS, 10 months; n = 23), which again raised the question on whether the observed radiographic effect was secondary to decreased vascular permeability and edema (a ''steroid-like'' effect) as suggested in other studies or to a real antitumor effect with potential effect on survival. Another question is whether the strict safety criteria, which led many responding patients to be taken off treatment, played a role in the discrepancy between response and survival. Such issues are being addressed by larger confirmatory studies; combinations of bevacizumab with other chemotherapy agents and schedules are equally being explored. These trials might also provide information on whether metronomic cytotoxic chemotherapies are better options for combination with antiangiogenic agents as proposed by some authors (75).

Protein Kinase C-β

Activated protein kinase C-β is a major signaling molecule in the VEGF signaling cascade and has been implicated in the angiogenesis in several tumors, including glioblastomas (76). Preclinical data in glioblastoma models have suggested that inhibition of protein kinase C-β by enzastaurin (LY317615) can target not only angiogenesis but also tumor cells directly (77). Preliminary results of a phase II study of enzastaurin in recurrent high-grade gliomas have been presented (Table 3). Among the 63 patients with glioblastoma, 14 achieved objective radiographic response and 3 others were stable for more than 3 months. Exposure to the drug was significantly lower in patients on enzyme-inducing anticonvulsants. Treatment was well tolerated, although thrombocytopenia and nonfatal intratumoral bleeds occurred in some patients (78). Based on these preliminary results, further studies using enzastaurin as single agent in escalated doses or in combination with temozolomide and radiotherapy are being planned or are ongoing.

Conclusions

The first molecular targeted therapy phase II clinical trials in malignant glioma have not translated into significant changes in current clinical practice, and to date, no new molecule seems to be promising enough as to justify a large phase III trial. The next generation of trials is exploring the possibility of addressing multiple targets through use of multitargeting single agents, combinations of single-targeting agents, and combination with cytotoxic chemotherapy and/or radiotherapy. Correlative studies linked to such trials are becoming increasingly sophisticated and comprehensive and hold the promise to revive initial hopes for a revolution in the field.

Not everything is difficult in malignant gliomas. The current standard treatment for malignant gliomas (chemoradiotherapy with temozolomide; ref. 1) is associated with very low toxicity and is relatively easy to combine with other drugs. This provides the unique opportunity of shifting research focus from recurrent to newly diagnosed disease. Such a shift in paradigm may provide more reliable correlative studies not only because trials in newly diagnosed disease may achieve increased response rates (and thus increase statistical power for correlative analyses) but also because the tissue available for such studies truly represents the current molecular status of the tumor. Better designed randomized phase II trials testing new compounds associated with standard therapy versus standard therapy alone in newly diagnosed patients are becoming the norm; enrolling patients into such trials is easier because all patients receive an effective treatment. Such randomized phase II trials are also being used to optimize treatment combinations through the use of multiple arms using slightly different doses and timing of treatments. Furthermore, surgical arms that allow for pharmacodynamic evaluation are becoming increasingly common and may boost our understanding of the *in vivo* mechanisms of

drug sensitivity and resistance. Prospective selection or exclusion of patients based on molecular characteristics is also becoming a reality and will provide insight on whether providing individualized, ''molecularly tailored'' therapies is a realistic goal in gliomas. This review focused on the development of new targeted chemical compounds but several other targeted strategies are being explored, including monoclonal antibodies, immunoconjugates, ribozymes, antisense oligonucleotides, RNA interference, gene therapy, and stem cell–based techniques. Hopefully, all this accumulating knowledge will translate into real benefit for glioma patients in the near future as witnessed in other types of tumors.

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Lessons learned in the development of targeted therapy for malignant gliomas

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