Chemotherapy for Glioblastoma: Current Treatment and Future Perspectives for Cytotoxic and Targeted Agents

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Abstract. Glioblastoma is the most frequent and devastating primary malignant brain tumor in adults. Surgery followed by standard radiotherapy with concomitant and adjuvant chemotherapy with temozolomide is the standard of care in patients with glioblastoma, however the prognosis remains poor with a median survival in the range of 12-15 months. Common genetic abnormalities in glioblastoma are associated with aberrant activation or suppression of cellular signal transduction pathways and resistance to radiation and chemotherapy. Special attention has been focused on targets such as epidermal growth factor receptor, vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and on pathways such as the phosphatidylinositol-3kinase/Akt/mammalian target of rapamycin and Ras/Raf/mitogen-activated protein-kinase pathways. Several signal transduction inhibitors have been examined in preclinical and clinical malignant glioma trials, including antiangiogenic agents (bevacizumab, enzastaurin), and inhibitors of epidermal growth factor receptor tyrosine kinase (gefitinib and erlotinib), mammalian target of rapamycin (temsirolimus, everolimus) and integrin (cilengitide). Although preliminary clinical results of the use of targeted agents have not translated into significantly better survival, more recent phase II trials are exploring the combination of multitargeted drugs with cytotoxic chemotherapy and radiotherapy in order to overcome the resistance of tumors to single-agent targeted therapies. This review summarizes the current results with cytotoxic and targeted molecular agents in glioblastoma and the development of new chemoradiation strategies under evaluation to increase their effectiveness.

Approximately 176,000 cases of central nervous system (CNS) cancer are diagnosed per year worldwide, with an estimated annual mortality of 128,000 (1). Gliomas are the most frequent intracranial tumor and account for more than 50% of all primary brain tumors, with glioblastoma (GBM) being by far the most common and aggressive (2, 3). Treatment of GBM includes surgery, radiotherapy (RT) and chemotherapy. Currently, surgery followed by standard RT with concomitant and adjuvant chemotherapy with temozolomide is the standard of care in patients with GBM aged <70 years, however the prognosis remains poor, with a median survival of 12-15 months (4). Clearly, there is a desperate need for more effective therapies for patients with GBM.

Overexpression, activation, and dysregulation of various membrane receptors, signaling pathways, and other factors occur frequently in GBM (5-7). Therapeutic approaches targeting these molecules, including inhibitors of epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), platelet-derivated growth factor receptor (PDGFR), and mammalian target of rapamycin (mTOR) have been examined in preclinical and clinical trials for malignant glioma. The efficacy of these agents as monotherapies has been modest, at best; however, new multitargeted kinase inhibitors and combinations of single-targeted kinase inhibitors in combination with radiation and cytotoxic chemotherapy will likely play an increasing role in the management of GBM and several randomized/prospective studies are ongoing. We have performed a review of the current results with chemotherapy in malignant glioma and of the new treatment strategies that may improve the prognosis for these patients.
Current Standard Therapy for GBM

Temozolomide is a second-generation imidazo-tetrazine derivative, which exerts its cytotoxic effects by methylation of specific DNA sites. The methylation of the O\(^6\) position of guanine in DNA is usually regarded as the most critical. Temozolomide is taken orally and is absorbed rapidly, with a nearly complete bioavailability after oral administration, across the blood-brain barrier, and achieves high concentration in the cerebrospinal fluid.

The recently published randomized European and Canadian trial (EORTC 26981/22981-NCIC) (4) has clearly demonstrated that the addition of temozolomide to RT, followed by 6 monthly cycles of temozolomide provides significant survival benefit with minimal additional toxicity in patients with GBM. The reported median survival was 14.6 months with RT plus temozolomide and 12.1 months with RT alone, with respective 2-year survival rates of 27% and 10%. The 2-year survival rate improved from 10.4% for RT alone to 26.5% in the RT plus temozolomide group. A recent up-date of the trial reported the final results, with a median follow-up of more than 5 years (8). Overall survival was 27.2% at 2 years, 16.0% at 3 years, 12.1% at 4 years, and 9.8% (6.4-14.0) at 5 years in RT plus temozolomide group, versus 10.9%, 4.4%, 3.0%, and 1.9% in the radiotherapy alone group (p<0.0001). A benefit of combined therapy was recorded in all clinical prognostic subgroups, including younger age, extent of resection, and good performance status. Methylation of the O\(^6\)-methylguanine-DNA-methyltransferase (MGMT) promoter was the strongest predictor of outcome and benefit from temozolomide chemotherapy.

The cytotoxic effect of temozolomide is correlated to the intracellular levels of MGMT. MGMT is a critical DNA repair protein that protects tumor cells against alkylating chemotherapeutic agents, transferring the methyl group to an internal cysteine acceptor residue. High levels of MGMT activity in tumor tissue are associated with resistance to temozolomide (9). Thus, epigenetic silencing of the MGMT DNA-repair gene by promoter methylation results in decreased levels of MGMT and may enhance temozolomide sensitivity, whereas unmethylated MGMT promoter is associated with potential temozolomide resistance (8, 10, 11). Hegi et al. (10) reported a survival advantage in GBM patients with methylated MGMT promoter treated by temozolomide and standard radiotherapy, whereas GBM patients with unmethylated MGMT promoter did not seem to have a survival benefit by chemoradiation combination. The 2-year survival rates for patients with unmethylated and those with methylated MGMT promoter treated with standard RT and temozolomide were 14% and 46%, respectively.

The use of standard or hypofractionated RT plus concomitant and/or adjuvant temozolomide has been recently extended to elderly people with GBM. The reported median progression-free survival and median survival durations were in the range of 7-9 months and 10-14 months, respectively, which compare favourably with series using RT alone (12-14). In 32 elderly patients of 70 years or older treated with standard RT and temozolomide given concomitantly and adjuvantly, the median survival was 10.8 months and the median progression-free survival was 6.7 months (12). The 12-month survival and progression-free survival rates were 36% and 16%, respectively. Longer survival has been reported for elderly patients who had MGMT promoter methylated (14).

Prolonged schedules with higher cumulative dose of temozolomide have been explored in order to maximize MGMT depletion in cancer cells and possibly to increase antitumor efficacy (15-17). Regimens using temozolomide administered 1 week on/1 week off or 21 days on/7 days off in patients with recurrent malignant glioma result in a more protracted MGMT depletion in blood cells and possibly in brain tumor tissue.

Wick et al. (16) reported on 90 patients with recurrent malignant gliomas treated with temozolomide (150 mg/m\(^2\)) administered 1 week on and 1 week off, showing a promising median progression-free survival of 24 weeks, being 43.8% at 6 months. The RTOG 052/EORTC 26052/220535 trial, led by the Radiation Therapy Oncology Group (RTOG) along with the European Organisation for Research and Treatment of Cancer (EORTC) and the North Central Cancer Treatment Group, which has enrolled 1,150 patients (closed in June 2008), is evaluating if a dose-intensity schedule of temozolomide (75 to 100 mg/m\(^2\) days 1 to 21 every 28 days maximum) improves survival compared with standard chemotherapy regimen. Currently, in the absence of robust data, the use of dose-intense temozolomide schedules as alternatives to standard regimens are not recommended in patients with GBM.

In summary, temozolomide given concomitantly and adjuvantly with RT after surgical resection represents the standard of care in patients with GBM. The epigenetic silencing of MGMT by promoter methylation is correlated with improved survival in patients treated with temozolomide. Although the prolonged exposure of cancer cells to temozolomide may represent a promising strategy to overcome resistance mediated by MGMT, at present, the clinical relevance of the use of a dose-dense temozolomide schedule remains to be proven.

Targeted Therapies

GBM is characterized by several aberrantly activated signaling pathways. Several growth factor receptors, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR) and platelet-derivated growth factor receptor (PDGFR), are overexpressed, amplified and/or mutated, leading to uncontrolled cell proliferation, angiogenesis, migration, survival and differentiation (18, 19) (Figure 1). Thus, several targeted agents are being developed...
as potential inhibitors of growth factor receptor transduction pathways and angiogenesis (Table I). The major challenge in the use of targeted therapies is the identification of the optimal therapeutic targets, and to select new agents which can translate to survival benefit for patients with GBM. Currently, the use of small molecule tyrosine kinases inhibitors (TKIs) and monoclonal antibodies against EGFR and VEGFR have been evaluated in phase II clinical trials as main anti-growth factor receptor strategies. Other potentially useful agents for the treatment of GBM are represented by the PDGFR-, mammalian target of rapamycin (mTOR) and integrin inhibitors. A summary of main recent phase II clinical trials of targeted therapies alone or in combination with other treatment modalities in GBM are shown in Table II.

**EGFR Inhibitors**

EGFR is a 170-kDa receptor tyrosine kinase member of the ErbB family which consists of four distinct receptors: HER1/EGFR (epidermal growth factor receptor), HER2, HER3 and HER4. It is formed of three major domains: the extracellular domain, the transmembrane domain and the cytoplasmatic domain, which harbors the tyrosine kinase activity. Phosphorylation of the tyrosine kinase domain activates several signaling pathways, such as the phosphatidylinositol 3'-kinase (PI3K)/Akt/mTOR, and Ras/mitogen-activated protein kinase (MAPK) (20, 21). Activation of EGFR pathways results in several biological processes, including cell proliferation, angiogenesis, migration, survival and differentiation.
Table I. Main molecular targeted agents used in the treatment of glioblastoma.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of action</th>
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<tbody>
<tr>
<td>EGFR inhibitors</td>
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<tr>
<td>Cetuximab</td>
<td>EGFR blocker (monoclonal antibody)</td>
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<tr>
<td>Gefitinib</td>
<td>EGFR TKI</td>
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<tr>
<td>Erlotinib</td>
<td>EGFR TKI</td>
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<tr>
<td>Lapatinib</td>
<td>EGFR, Erb-2 TKI</td>
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<td>Canertinib</td>
<td>Erb-1, Erb-2, Erb-3, Erb-4 TKI</td>
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<tr>
<td>Pelitinib</td>
<td>Irreversible EGFR inhibitor</td>
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<tr>
<td>BIBW-2992</td>
<td>EGFR and HER2 inhibitor</td>
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<tr>
<td>PDGFR</td>
<td></td>
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<tr>
<td>Imatinib mesylate</td>
<td>PDGFR TKI, c-Kit, Bcr-Abl</td>
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<tr>
<td>Vandetinib</td>
<td>PDGFR, FLT3 (FMS-like tyrosine kinase-3), c-Kit inhibitor</td>
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<tr>
<td>VEGFR inhibitors</td>
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<tr>
<td>Bevacizumab</td>
<td>VEGFR blocker (monoclonal antibody)</td>
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<tr>
<td>VEGF trap</td>
<td>Pan VEGF blocker</td>
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<tr>
<td>Vatalanib</td>
<td>VEGFR-1, VEGFR-2, PDGFR, c-Kit TKI</td>
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<tr>
<td>Vandetanib</td>
<td>EGFR, HER2 inhibitor</td>
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<tr>
<td>Cediranib</td>
<td>Pan-VEGFR, PDGFR, C-Kit TKI</td>
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<tr>
<td>Sunitinib</td>
<td>VEGFR, PDGFR, c-Kit TKI</td>
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<tr>
<td>Pazopanib</td>
<td>VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, c-Kit TKI</td>
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<tr>
<td>mTOR inhibitors</td>
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<tr>
<td>Sirolimus</td>
<td>mTOR inhibitor</td>
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<tr>
<td>Temsirolimus</td>
<td>mTOR inhibitor</td>
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<tr>
<td>Everolimus</td>
<td>mTOR inhibitor</td>
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<tr>
<td>AP23573</td>
<td>mTOR inhibitor</td>
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<tr>
<td>PKC inhibitors</td>
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<tr>
<td>Enzastaurin</td>
<td>PKC inhibitor</td>
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<tr>
<td>RAF-MEK-ERK inhibitors</td>
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<tr>
<td>Tipifarnib</td>
<td>Farnesyltransferase inhibitor</td>
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<tr>
<td>Lonafarnib</td>
<td>Farnesyltransferase inhibitor</td>
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<tr>
<td>Sorafenib</td>
<td>Inhibitor of farnesyltransferase I and VEGFR and PDGFR TK</td>
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<td>mTOR inhibitors</td>
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<td>Inteogens</td>
<td>αvβ3 and αvβ5 integrin inhibitor</td>
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EGFR, Epidermal growth factor receptor; VEGF, vascular endothelial growth factor; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; TKI, tyrosine kinase inhibitor.

EGFR amplification and/or overexpression occur in approximately half of GBM cases (22, 23). EGFRvIII is a mutant EGFR variant characterized by deletion of the extracellular ligand-binding domain, which results in a constitutionally active receptor and is reported in 20-40% of GBMs (24, 25). All these alterations are responsible for the activation of EGFR downstream signaling pathways, promote the oncogenic process, and are associated with a negative prognosis (26, 27). Moreover, in the clinical setting, EGFR overexpression in GBM has been associated with resistance to radiation therapy (28). Therefore, a large number of potentially therapeutic targets have been developed in order to block the EGFR signaling pathways, including monoclonal antibodies and small molecule TKIs.

Preclinical studies with the murine humanized monoclonal antibody cetuximab (Erbitux, Merck & Co) have shown that EGFR inhibition leads to apoptosis while reducing proliferation as well as VEGF expression in GBM cells (29). An additive cytotoxic effect can be observed in different GBM cell lines by the addition of cetuximab to radiochemotherapy with temozolomide (30). Currently a one-armed single-center phase I/II trial is evaluating the safety and efficacy of combined radiotherapy with temozolomide and cetuximab as first-line treatment for patients with primary GBM (31).

Gefitinib (Iressa, AstraZeneca) and erlotinib (Tarceva, Osi Pharmaceuticals) are two oral, small TKI molecules that have been evaluated in several phase I and II studies in patients with GBM. Phase I and II studies of gefitinib and erlotinib used as single agents adjuvant to RT have shown no major improvements in survival (32-38). In a phase II trial that included 53 patients with recurrent GBM, gefitinib was well tolerated, however, it resulted in a modest progression-free and median survival of 8.1 and 39.4 weeks respectively (33); similar results have been shown by others (29, 31, 32). Phase II trials of erlotinib single-agent therapy in recurrent GBM have demonstrated a similar median progression-free survival of only 2-3 months, with response rates of 6-25% (37-39). In a recent randomized phase II trial (EORTC Brain Tumor Group Study 26034) of erlotinib versus temozolomide or carmustine in 110 patients with recurrent GBM, the 6-month progression free survival rate was 11.4% in the erlotinib arm and 24% in the control arm (40). The reported clinical evidence clearly suggests that the use of EGFR inhibitors erlotinib and gefitinib as single agents in patients with progressive GBM is not beneficial.

There is little evidence that erlotinib and gefitinib may be associated with survival advantages in a subgroup of patients with GBM who have overexpressed, amplified, or mutated EGFR in association with intact PTEN or unphosphorylated Akt (41, 42). Mellinghoff et al. (42) reviewed the molecular characteristics of 26 patients with malignant gliomas treated with EGFR TKIs. Coexpression of EGFRvIII and PTEN, a tumor-suppressor protein that inhibits the PI3K signaling pathway, was significantly associated with better clinical response to EGFR TKIs. Loss of PTEN may promote resistance to EGFR kinase inhibitors. In contrast, Lassman et al. (43) found no correlation between sensitivity to erlotinib or gefitinib and changes in expression, amplification, phosphorylation, downstream signaling of EGFR, and genomic changes.

More recently, phase I/II studies testing the combination of erlotinib in association with RT and/or temozolomide showed conflicting results (44-46). Using this regimen, Prados et al. (46) showed a median progression-free survival...
of 8.2 months and median survival of 19.3 months. Compared with the EORTC 26981/22981-NCIC conducted by Stupp et al. (4), where patients were treated with RT and temozolomide alone, the study showed survival benefit, especially in patients with MGMT promoter methylation and PTEN positivity. By contrast, a recent study of the North Central Cancer Treatment Group (45) using a similar regimen showed no significant additional benefit for erlotinib when combined with RT and temozolomide, and no differences in survival for patients characterized by EGFRvIII plus wild-type PTEN compared with patients without these genotypic features. Moreover, the regimen was associated with significant toxicity, including two grade 5 toxicities (non-neutropenic pneumonias). To date, the potential role of erlotinib in association with temozolomide and RT remains unclear.

New growth factor receptor inhibitors are emerging as potential therapeutic agents in GBM. Lapatinib (Tykerb, Glaxo Smith-Kline) is a dual EGFR and ErbB-2 inhibitor that has shown some activity in the treatment of solid
tumors, especially of advanced or metastatic breast cancer (47). Canertinib (Pfizer, New York) is a pan-ErbB inhibitor which has shown potent and sustained inhibition of tyrosine kinase activity selective for erbB1, erbB2, erbB3, and erbB4 in human cancer cell lines (48). Treatment of athymic nude mice bearing xenografts of human SF-767 glioblastoma with canertinib resulted in highly significant suppression of tumor growth (49). However, no clinical data are available in patients with high-grade gliomas.

In summary, the patterns of response observed in patients treated with EGFR TKIs are disappointing. Despite EGFR activating several signaling pathways, such as PI3K/Akt/mTOR, and Ras/MAPK, it is possible that other growth factor and signaling pathways play a greater role in maintaining the transformed phenotype of GBM. Erlotinib in association with RT and temozolomide has led to modest or no survival benefit compared to RT plus temozolomide alone, and currently its use is not advisable in clinical practice. New strategies include the combination of EGFR TKIs with targeted agents able to inhibit other signaling pathways, and phase II trials testing the association with temsilorus and sorafenib are ongoing (http://clinicaltrials.gov/ct2/show/NCT00474786).

Platelet-derived Growth Factor Inhibitors

Imatinib mesylate (Gleevec, Novartis) is an inhibitor of the kinase activity of multiple growth factor receptors, including PDGFR, c-Kit, and Bcr-Abl. The PDGF family consists of four members, PDGF-A, -B, -C and -D, which signal through the alpha and beta PDGF receptor (PDGFRα and PDGFRβ) tyrosine kinases. Binding of ligands induces dimerization and cross-tyrosine phosphorylation of the intracellular domain. This results in the activation of PI3K, MAPK, Jak family kinase, Src family kinase, and phospholipase C-gamma (PLC-γ) signal transduction pathways. Overexpression of alpha subtype receptor (PDGFRα) has been reported in approximately one third of primary glioblastoma (50). c-Kit is a receptor tyrosine kinase that acts similarly to EGFR and PDGFR and is overexpressed in GBM. c-Kit consists of an extracellular domain, a transmembrane segment, a juxtamembrane segment, and a protein kinase domain. Binding of stem cell factor to Kit results in receptor dimerization and activation of several signal transduction pathways including Akt, Src family kinases, PI3K, PLC-gamma, and Ras/MAPK.

Imatinib mesylate used as single agent in the dose range of 600 to 1,000 mg/d is generally well tolerated but shows limited activity in patients with recurrent GBM (51, 52). In a small phase I/II study of the North American Brain Tumor Consortium (51) including 34 patients with recurrent GBM, the 6-month progression-free survival was 3%, with only 2 and 6 patients who had partial response and stable disease, respectively. In a recent phase II EORTC study including 51 patients with recurrent GBM, Raymond et al. (52) reported a 6-month progression-free survival of 16% with 3 patients who had an objective partial response. Imatinib mesylate plus hydroxyurea is associated with better antitumor activity than imatinib given alone. At a median follow-up of 58 weeks, Reardon et al. (53) reported a median progression-free survival of 14.4 weeks and 6-month progression-free survival of 26% in 33 patients with recurrent GBM. Nine percent of patients achieved radiographic response, and 42% of patients achieved stable disease. The most common toxicities included grade 3 neutropenia in 16% of patients, thrombocytopenia in 6% of patients, and edema in 6% of patients. In a phase I study of 55 patients with GBM, the combination of imatinib plus temozolomide was well tolerated (54). Grade 3 or 4 hematological toxicities occurred in fewer than 10% of patients. The most common nonhematological toxicities included grade 2 nausea/emesis and fatigue in 5% of patients. Overall survival and progression-free survival were 45.1 and 26.6 weeks, with a 6-month survival of 53%. In summary, in the dose range of 600 to 1,000 mg/d, single-agent imatinib is well tolerated but has limited antitumor activity in patients with recurrent GBM, and available data do not support its use in clinical practice. Combination regimens incorporating imatinib are encouraging and phase II trials have been planned.

VEGF/VEGFR Inhibitors

Vascular proliferation, or neoangiogenesis, is a distinct histopathological characteristic of GBM and is correlated with prognosis (55-57). VEGF is a key factor involved in the angiogenic process that can elicit several responses such as endothelial cell proliferation, extracellular matrix degradation, cell migration, and expression of other proangiogenic factors (matrix metalloproteinase-1, urokinase-type plasminogen activator and its receptor, plasminogen activator inhibitor-1). VEGF expression is stimulated by hypoxia, acidosis, and many growth factors as EGF, PDGF, hepatocyte growth factor (HGF), c-Kit and their downstream signaling pathways (PI3K-Akt, Ras-MAPK). The main receptors involved in relaying VEGF-A signaling are VEGFR-1 and VEGFR-2.

The rationale for anti-VEGF/VEGFR therapies has been supported by several in vitro and in vivo studies (58, 59). Bevacizumab (Avastin, Genentech) is a humanized murine monoclonal antibody that selectively blocks VEGF, preventing the activation of VEGF receptor tyrosine kinases VEGFR1 and VEGFR2 (60). In patients with metastatic colorectal cancer and recurrent non-small cell lung cancer (NSCLC), bevacizumab in combination with conventional chemotherapy resulted in longer survival (61, 62). Phase II studies in patients with recurrent GBM using bevacizumab alone or in association with irinotecan have been reported (63-69) (Table II). Vredenburgh et al. (63) reported on 35 patients with recurrent GBM treated
with bevacizumab plus irinotecan. Twenty-three patients received bevacizumab at 10 mg/kg plus irinotecan every 2 weeks and 12 patients received bevacizumab 15 mg/kg every 21 days and irinotecan on days 1, 8, 22, and 29 for a total of 6 cycles. The 6-month progression-free survival and overall survival rates were 46% and 77%, respectively. Fifty-seven percent of patients had at least a partial radiological response. Kreisl et al. (64) reported on 48 patients with recurrent GBM treated with bevacizumab followed by bevacizumab plus irinotecan at progression of tumor. The median survival and progression-free survival were 31 weeks and 16 weeks, with a respective 6-month survival and progression-free survival of 57% and 29%. Thirty-five percent of patients achieved a radiographic response. In contrast, amongst 19 patients treated with bevacizumab plus irinotecan at progression, the median progression-free survival was only 30 days, with no objective radiographic responses. Thromboembolic events (12.5%), hypertension (12.5%), hypophosphatemia (6%), and thrombocytopenia (6%) were the most common drug-associated adverse events. Similar 6-month progression-free and overall survival, and discontinuation of steroids for recurrent GBM to a bevacizumab-based treatment have been reported by others (65-68), suggesting that bevacizumab improves local control and survival in this patient population.

Based on this clinical evidence, more recently the US Food and Drug Administration (FDA) Oncologic Drugs Advisory Committee (ODAC) has approved the use bevacizumab as a single agent for patients with GBM, with progressive disease following prior therapy.

The feasibility of using bevacizumab with chemoradiation in the primary management of GBM has been tested in few studies (69, 70). Narayana et al. (70) treated 15 patients with standard RT and bevacizumab at the dose of 10 mg/kg on days 14 and 28 and daily temozolomide (75 mg/m²). Subsequently, bevacizumab at the same dose was continued every 2 weeks with temozolomide (150 mg/m²) for 12 months. Radiographic responses were noted in 13 of 14 assessable patients (93%). One-year progression-free survival and overall survival rates were 59% and 87%, respectively. Four patients had grade III/IV nonhematologic toxicities. These positive outcomes have led to the initiation of a new phase III trial (RTOG-0825) testing concurrent chemoradiation and adjuvant temozolomide plus bevacizumab versus conventional chemoradiation and adjuvant temozolomide in patients with newly diagnosed GBM.

Several new antiangiogenic inhibitors have been recently developed, including vatalanib (Novartis Pharmaceutical), cediranib (Recentin, Astra Zeneca), sunitinib (Sutent, Pfizer), sorafenib (Nexavar, Bayer Pharmaceuticals), vandetanib (Zactima, Astra Zeneca), and VEGF trap (Afiblercept, Regeneron Pharmaceuticals). These targeted agents have demonstrated antitumor activity in preclinical glioma models, alone or in combination with RT (71-78), and are currently undergoing clinical evaluation in GBM.

Vatalanib is an oral VEGFR-1 and VEGFR-2 TKI that is able to inhibit VEGFR, PDGFR, c-Kit and colony-stimulating factor 1 receptor (CSF1R). It has been recently tested in two phase I/II studies given alone or in combination with cytotoxic chemotherapy (79-80). In a study of 47 patients with recurrent GBM treated with vatalanib as single agent, a partial response was shown in 4% and stability of disease in 56% of patients (79). Reardon et al. (80) reported the results of vatalanib in association with either temozolomide or lomustine in 51 patients. The progression-free survival was 16 weeks in vatalanib plus temozolomide group and 10 weeks in vatalanib plus lomustine group. A recent phase I trial from the same authors has shown that vatalanib at doses up to 1000 mg twice-a-day combined with imatinib and hydroxyurea were well tolerated (81).

Cediranib is an oral pan-VEGFR TKI that also inhibits PDGFR and C-Kit. Batchelor et al. (82) reported the results of cediranib used as single agent in 16 patients with recurrent GBM, showing a promising median overall survival of 7 months and median progression-free survival of 3.5 months. Magnetic resonance imaging (MRI) imaging showed a significant decrease in tumor enhancement in more than 50% of patients. Contrast enhancement decreased after a single dose of cediranib in all patients, and this was associated with a significant reduced vasogenic edema. Toxicity was modest and included diarrhea, dysphonia, and hypertension. A phase III randomized trial comparing the efficacy of cediranib monotherapy or the combination of cediranib with lomustine to the efficacy of lomustine alone is planned (http://clinicaltrials.gov/ct2/show/NCT00777153).

Sunitinib and sorafenib are two multitarget TKIs that have demonstrated potent antitumor and antiangiogenic activity in preclinical models (72, 75, 77, 78). Sunitinib is a small, orally bioavailable molecule which is able to inhibit PDGFR-α and PDGFR-β, as well as VEGFR-1 and -2, and c-KIT. Sunitinib has demonstrated efficacy and tolerability in several phase II/III clinical trials involving patients with gastrointestinal stromal tumor, metastatic renal cell carcinoma, and is currently tested in breast cancer and NSCLC (83-85). There are no clinical data in patients with GBM; however, phase I/II studies of Sunitinib as single-agent (http://clinicaltrials.gov/ct2/show/NCT00713388) or in combination with irinotecan (http://clinicaltrials.gov/archive/NCT00611728) are ongoing. Sorafenib is one of the most promising agents targeting PDGFR, VEGFR, and Raf and has been recently approved by the Food and Drug Administration for the treatment of renal cell carcinoma (86-88). Strategies to antagonize PKC and ERK pathways in combination seem to be an attractive approach to enhance therapeutic efficacy in human glioma cells also, and a phase II evaluation of sorafenib in glioma as a single agent and in
combination with temozolomide or erlotinib is ongoing (http://clinicaltrials.gov/ct2/show/NCT00445588).

Vandetanib is an inhibitor of VEGFR-2 tyrosine kinase with additional effects on EGFR-3 and EGFR (89, 90) that has shown significant antitumor effects in combination with radiotherapy in rat glioma models (91). Phase I/II studies are exploring the use of vandetanib alone or in combination with chemoradiation in patients with malignant glioma (http://clinicaltrials.gov/ct2/show/NCT00821080 and /NCT00613223), however, currently no clinical data are available.

VEGF Trap is a 110 kDa soluble protein that is able to bind circulating VEGF, inhibiting its activity (71), and to potentiate RT in preclinical GBM xenografts (92). De Groot et al. (93) reported the results of a phase II trial of VEGF Trap monotherapy for 48 patients with recurrent GBM and anaplastic glioma at first relapse. Response rates were 50% for the anaplastic glioma group and 30% for the glioblastoma group. Grade 3 toxicity occurred in 40% of patients and included fatigue, hypertension, hand-foot syndrome, lymphopenia, thrombosis and proteinuria, causing the discontinuation of therapy in 25% of patients.

In summary, VEGF inhibitors target one important pathway in brain tumors. Bevacizumab represents the first antiangiogenic agent approved for use in malignant glioma. In addition to bevacizumab, there is an array of inhibitors of VEGF, VEGFR, and other relevant targets that could be effective in selected patients with GBM. Combining these antiangiogenic agents with conventional chemotherapy or radiation is a promising antitumor strategy that is currently being investigated in several phase II clinical trials with the intent to produce a synergistic antitumor effect and a survival benefit for patients with GBM, targeting both the tumor cell and tumor vascular compartment. Certainly, an improved understanding of resistance to antiangiogenic therapy is necessary to identify effective strategies for the patients that progress despite treatment.

Mammalian Target of Rapamycin Inhibitors

Overactivation of the PI3K/Akt/mTOR pathway seems to play an important role in gliomagenesis (94). Combined activation of Ras and Akt leads to the formation of GBM in mice (95). In human GBM, Akt is activated in approximately 70% of these tumors, in association with loss of PTEN and/or activation of EGFR and PDGFR tyrosine kinases. Alterations of PTEN expression are present in 20–40% of GBM (96, 97), and have been associated with a worse prognosis, although conflicting results have been reported (97-99). There is emerging evidence suggesting that mTOR is a critical downstream component in PTEN/Akt signaling (100-103), and pharmacological inactivation of mTOR reduces neoplastic proliferation and tumor size in PTEN-deficient mice (104). These evidence has provided the rationale for clinical studies of mTOR inhibitors in GBM.

Temsirolimus (Torisel, Wyeth Pharmaceutical), everolimus (Certican, Novartis Pharmaceutical) and AP23573 (ARIAD Pharmaceutical) are three synthetic analogs of rapamycin (sirolimus) currently being tested as potential targeted drugs in GBM. Results of phase II studies using temsirolimus as single agent found only a limited activity in recurrent GBM (105, 106). More recently, based on evidence of the synergism between mTOR inhibitors and EGFR TKI (107), ongoing trials have been exploring such a combination (108, 109). A phase I study found no significant pharmacokinetics interaction between sirolimus and gefitinib, with partial radiological response and stable disease that were seen only in 2 and 13 of 34 patients, respectively (108). Similarly, a study combining gefitinib and everolimus failed to find survival benefit of this regimen in comparison with historical controls, although a radiological response occurred in about one third of patients (109).

Despite these preliminary disappointing results, several studies are exploring the safety and efficacy of mTOR inhibitors in combination with other targeted agents and/or chemoirradiation. Ongoing phase II studies are assessing the maximum tolerable dose and the efficacy of temsirolimus plus sorafenib (http://www.cancer.gov/clinicaltrials/NCT00536433), temsirolimus plus sorafenib and erlotinib (http://clinicaltrials.gov/ct2/show/NCT00335764), and temsirolimus or everolimus plus irradiation (http://groups.eortc.be/brain/html/trials/26082-22081) or chemoradiation with temozolomide (http://clinicaltrials.gov/ct2/show/NCT00553150).

In summary, although data strongly support the view of the PTEN/PI3K/AKT pathway as an important target for drugs, current clinical results on the use of mTOR inhibitors remains disappointing. A new generation of trials is seeking to define whether the combination of two or more targeted drugs together with RT and cytotoxic chemotherapy can overcome tumor resistance.

Protein Kinase C, RAF-MEK-ERK, and Integrin Inhibitors

Enzastaurin is an oral serine/threonine kinase inhibitor that selectively inhibits PKC-β, a mediator of VEGF intracellular signaling. Unfortunately, a phase III study comparing lomustine with enzastaurin in recurrent GBM was discontinued at the first interim analysis of 266 patients due to lack of survival benefit of enzastaurin over the control group (109). As for other targeted agents that failed to show tumor activity when used as single agents, combinations of enzastaurin with RT (110-111) or temozolomide (http://www.eortc.be/protocol/26054) are currently being investigated in phase I/II studies.
The RAF-MEK-ERK signal transduction pathway is an important mediator of dysregulated glioma cell proliferation and angiogenesis. Tipifarnib (Zarnestra, Johnson and Johnson), lonafarnib (Sarasar, Shering-Plough), and sorafenib (Nexavar, Bayer) may inactivate RAS by inhibiting farnesyltransferase. A phase II study conducted in 22 patients with recurrent malignant glioma treated with tipifarnib (300 mg twice a day for 21 days every 4 weeks) showed modest evidence of activity, with a 6-month progression-free survival of 12% (112). Combination of these agents in association with radiotherapy and chemotherapy has been reported in few studies (113, 114). In a phase I study of 13 patients with GBM, tipifarnib (200 mg/day) concurrent with standard RT was well tolerated and resulted in a median survival of 12 months (113). Lonafarnib in combination with temozolomide resulted in a 6-month progression-free survival of 38% in 23 patients with recurrent GBM (114). Phase I/II studies on the combination of tipifarnib, lonafarnib, and sorafenib in association with RT, temozolomide and other targeted agents (http://clinicaltrials.gov/ct2/show) are ongoing, however no clinical results are currently available.

Integrins are heterodimeric cell surface adhesion receptors that consist of two noncovalently associated alpha and beta subunits, and function as receptors for extracellular matrix proteins such as fibronectin, laminins, collagens, and vitronectin (115). Ligand binding to the extracellular domain of integrin receptors results in receptor activation and in the transduction of signals essential for cell adhesion, migration, proliferation, differentiation, and survival (116). The integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ appear to be particularly important in the process of angiogenesis and are expressed in a variety of malignancies, including gliomas. The critical role of integrins in angiogenesis and association with tumor progression make them an attractive target for anticancer therapy. Cilengitide (EMD 121974), a cyclic Arg-Gly-Glu (RGD) peptide is a potent and selective inhibitor of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (115-118). A phase II study has been conducted in 81 patients with recurrent GBM randomly assigned to receive either 500 or 2,000 mg of cilengitide twice weekly on a continuous basis (119). Cilengitide was well tolerated, with no significant toxicities observed in either arm. A modest antitumor activity was observed in both treatment groups, with a 6-month progression-free survival of 15% and a median overall survival of 9.9 months, which were more favorable among patients treated with 2,000 mg. In a phase I/II trial of RT with concomitant and adjuvant cilengitide and temozolomide, Stupp et al. (120) reported a 6-month progression-free survival of 69% and a 12-month survival of 67%, with no added toxicity. A phase III EORTC 26071-22072 study is testing cilengitide in combination with standard treatment (standard RT with concomitant and adjuvant temozolomide) versus standard treatment in patients with newly diagnosed GBM with methylated MGMT promoter gene.

**Conclusion**

The current standard treatment for GBM is chemoradiotherapy with temozolomide following surgical resection. Identification of many molecular genetic and signal transduction pathways involved in oncogenesis have yielded many targeted drugs that are currently undergoing clinical evaluation in GBM. Currently, several drugs have been tested, including EGFR TKIs (gefitinib and erlotinib), antiangiogenic agents (bevacizumab, enzastaurin), and inhibitors of mTOR (temsirolimus, everolimus) and integrin (cilengitide). Although bevacizumab has been recently approved for use as a single agent for patients with GBM, with progressive disease, most of the molecular targeted therapy phase II clinical trials in GBM have not translated into significant survival advantages. Better molecular characterization of GBM could allow avoidance of negative results in large clinical trials enrolling heterogeneous cohorts of patients, and potentially allow the design of “individualized” therapies based on the genetic and molecular characteristics of each tumor. New chemotherapeutic strategies are represented by the combination of multitargeted drugs with cytotoxic chemotherapy and radiotherapy in order to overcome tumor resistance. Randomized clinical trials remain the gold standard for the evaluation of new targeted agents. Most multicenters randomized European (EORTC) and American (RTOG, NABTG) clinical trials are testing these new agents in combination with standard chemoradiotherapy (RT plus concomitant and adjuvant temozolomide) versus standard chemoradiotherapy alone. This is important because all the patients will be treated appropriately. Hopefully, results from these randomized trials and the development of new chemoradiation strategies will translate into real benefit for patients with GBM.

**References**


