Recent Advances in Therapy for Glioblastoma

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Glioblastoma is the most common primary malignant brain tumor in adults and is a challenging disease to treat. The current standard of care includes maximal safe surgical resection, followed by a combination of radiation and chemotherapy with temozolomide. Despite that, recurrence is quite common, and so we continue to search for more effective treatments both for initial therapy and at the time of recurrence. This article will review recent advances in therapy for glioblastoma, including surgery, radiotherapy, cytotoxic chemotherapies, molecularly targeted agents, and immunotherapy; the role of antiangiogenic agents in the treatment of glioblastoma is discussed in a separate article in this issue of the Archives.

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Approximately 51,000 primary brain tumors are diagnosed in the United States each year, 36% of which are gliomas. Of these, half are glioblastoma (GBM), or World Health Organization grade IV astrocytoma. Glioblastoma is the most aggressive form of glioma and, despite recent advances, continues to have a grim prognosis. The current standard of care for GBM begins with maximal safe surgical resection. After surgery, the combination of radiotherapy (RT) with temozolomide followed by adjuvant temozolomide therapy was shown to be significantly, although modestly, better than RT alone in a phase 3 clinical trial coordinated by the European Organization for Research and Treatment of Cancer and the National Cancer Institute of Canada. Median overall survival in the chemoradiotherapy arm was 14.6 months compared with 12 months in the RT arm. Perhaps more importantly, however, the percentage of patients alive at 2 years increased from approximately 10% to approximately 26%.

A post hoc analysis of tumor tissue in a subset of patients in the phase 3 trial demonstrated that patients whose tumors have methylation of the promoter region of the methylguanine methyltransferase (MGMT) gene (GenBank 4255) survived longer than those whose tumors were not methylated and on average derived greater benefit from the addition of temozolomide to RT. However, temozolomide provided modest benefit in the nonmethylated group, with borderline statistical significance. There is currently no proven alternative treatment for patients with nonmethylated tumors, so the combination of RT and temozolomide remains the treatment of choice for all patients with GBM at this time.

However, the median progression-free survival after RT with temozolomide and adjuvant temozolomide therapy is only 7 months, and a subset of patients' tumors show inexorable growth despite combined chemoradiotherapy. Therefore, we continue to search for more effective treatments to treat this difficult disease. This article will review recent advances in the treatment of GBM.

ADVANCES IN SURGICAL TREATMENT

Prospective surgical trials are very difficult to design and implement and, as such, few have been attempted. One recent multicenter phase 3 study from Germany evaluated the utility of 5-aminolevulinic acid (a fluorescent label) to assist surgeons in achieving a radiographic gross total resection of the contrast-enhancing portion of GBM. Only patients with ring-enhancing...
tumors that were believed to be potentially fully resectable were eligible to participate. All patients underwent maximal resection of tumor; they were randomized to the use of standard white light or fluorescence for intraoperative guidance. Use of 5-aminolevulinic acid allowed for a significantly higher rate of complete resection of enhancing disease on postoperative magnetic resonance imaging performed within 72 hours of surgery; gross total resection was achieved in 65% of patients in the treatment arm compared with 35% in the conventional surgery arm.

Further analysis of the data from this trial has demonstrated that patients who underwent gross total resection, regardless of the treatment arm, had superior survival to those who received subtotal resection. Although prospective data specifically addressing the issue of surgical extent of resection have never been collected, it is fairly well accepted at this point that cytoreduction via maximal safe resection improves survival. It also appears that use of 5-aminolevulinic acid intraoperatively may help to maximize resection.

ADVANCES IN RT

The standard of care for RT for GBM is focal, fractionated external beam RT (EBRT), but new techniques and technologies continue to be evaluated. Although no prospective, randomized studies have compared the 2 techniques, intensity-modulated RT (IMRT) is becoming widely used and appears to be fairly comparable to more traditional 3-dimensional EBRT. A number of dose-intensification techniques, such as brachytherapy, hyperfractionation, and the combination of EBRT with stereotactic radiation “boosts,” have been investigated, but none has been clearly shown to be superior to standard EBRT.

ADVANCES IN MEDICAL THERAPY

Treatment of GBM can be divided into 2 major situations: initial treatment and treatment at disease recurrence. New agents or treatment delivery techniques are typically tested first in the recurrent disease setting, where there are few approved treatment alternatives. Promising agents may then be combined with EBRT and temozolomide in the initial treatment setting because this is the established standard of care.

Alkylating Agents

Many GBMs have or develop resistance to alkylating chemotherapeutic agents such as temozolomide. One common mechanism of resistance is mediated by the enzyme encoded by the MGMT gene, O\(^6\)-alkylguanine-DNA alkyltransferase. Methylation of the promoter region of the gene silences it, leading to greater sensitivity to temozolomide. One promising strategy for overcoming resistance is simply administering more frequent temozolomide doses in what are referred to as dose-dense or dose-intense schedules. The Radiation Therapy Oncology Group has recently completed a phase 3 study randomizing patients between the standard 5-day regimen of temozolomide and a dose-intense 21-day monthly regimen of temozolomide after chemoradiotherapy; results are pending. Another strategy is direct enzyme inhibition using O\(^6\)-benzylguanine, which has been studied in combination with temozolomide. A second mechanism of resistance is mediated by the poly(adenosine diphosphate–ribose) polymerase (PARP) system; a number of PARP inhibitors have been developed and are being tested in early-phase clinical trials in combination with RT and temozolomide therapy.

Molecularly Targeted Agents

As we learn more about the biology of GBM and its aberrant signaling pathways, the neuro-oncology community has begun to investigate the role of molecularly targeted agents inhibiting these pathways (Figure). Most of the targeted agents are small-molecule tyrosine kinase inhibitors or monoclonal antibodies. The signaling pathways targeted include tumor growth factor pathways, angiogenesis pathways, and the intracellular signaling pathways that lie downstream of both. The angiogenesis pathways and their associated antiangiogenic agents are covered separately in this issue of the Archives.

A wide variety of targeted agents are being studied in the preclinical setting and in clinical trials. The overall experience at this point has been that monotherapy at recurrence with highly targeted tyrosine kinase inhibitors of all types has shown limited efficacy. It remains an open question for many of these agents whether molecular selection of tumors with particular mutations in the pertinent pathway may optimize efficacy; results of this type of strategy thus far have been mixed. Overall survival in multiple recent single-arm studies combining targeted agents with EBRT and temozolomide for first-line treatment has been modestly superior relative to historical controls. Because results with highly targeted agents have been somewhat disappointing, there has been a move toward combined inhibition of multiple targets, shutting down proximal and distal targets within the same pathway or shutting down targets in separate, parallel pathways. This can be accomplished via a combination of multiple agents or via single agents that inhibit multiple kinases. The potential for greater efficacy by inhibiting multiple pathways is counterbalanced by the corresponding increase in the risk of toxic effects from systemic inhibition of these same pathways.

Epidermal Growth Factor. Epidermal growth factor (EGF) and its receptor, EGFR, have been implicated in the growth of a number of tumors, including GBM. Binding of the EGF ligand to the extracellular portion of the EGFR activates the intracellular tyrosine kinase domain, triggering a variety of signaling cascades. Abnormally increased EGFR signaling activity is frequent in GBMs; it can be the result of overexpression due to polysomy or amplification or the result of mutation (eg, the EGFR-vIII mutant, seen in roughly 40% of GBMs, has a constitutively active tyrosine kinase domain owing to a deletion in the extracellular binding domain). Antibodies to EGFR such as cetuximab are currently under evaluation in early-phase clinical trials. The small-molecule EGFR inhibitor erlotinib hydrochloride has been more extensively evaluated as a single agent in recurrent dis-
ease and in combination with RT and temozolomide for initial treatment. Results have been disappointing in recurrent disease\textsuperscript{11} and mixed in initial treatment, with one study suggesting mildly improved survival\textsuperscript{12} but a second study failing to show improvement.\textsuperscript{13}

Additional studies to evaluate EGFR inhibitors in combination with other agents are ongoing, as are prospective studies to evaluate subpopulations that may be more likely to derive benefit from this class of agents. Two retrospective studies have found a correlation between activity of the protein kinase B/AKT pathway and erlotinib response in tumors with overexpression of EGFR, although this has not been confirmed prospectively.\textsuperscript{14,15}

**Platelet-Derived Growth Factor.** Platelet-derived growth factor (PDGF) and its receptor (PDGFR) are also commonly overactive in GBM, and, like activation of EGFR, activation of PDGFR triggers multiple intracellular signaling cascades. Imatinib mesylate is the best-known PDGFR inhibitor, although it also inhibits BCR-ABL and c-KIT. Phase 2 studies have evaluated imatinib in recurrent GBM as a single agent\textsuperscript{16} and in combination with hydroxyurea.\textsuperscript{17} but again, as with EGFR inhibitors, the results have been disappointing overall.

**Hepatocyte Growth Factor/Scatter Factor.** Hepatocyte growth factor (also known as scatter factor) binds to the c-MET receptor, activating intracellular signaling cascades similar to those triggered by EGFR and PDGFR; c-MET signaling is thought to be associated with invasion. In addition to stimulating c-MET, hepatocyte growth factor activates the EGF and vascular endothelial growth factor pathways. Multiple c-MET inhibitors are currently under evaluation; one, AMG102, is a human monoclonal antibody against hepatocyte growth factor that is currently in phase 2 study. Several small-molecule inhibitors are also currently under investigation.

**Phosphoinositide-3 Kinase/AKT/Mammalian Target of Rapamycin Pathway.** One of the intracellular second-messenger systems activated by EGFR, PDGFR, and the c-MET receptor is the phosphoinositide-3 kinase pathway, which leads to activation of AKT, also called protein kinase B, and then several targets further downstream, including the mammalian target of rapamycin. The major negative regulator of this pathway is the phosphate and tensin homologue (PTEN); there is frequently mutation of the PTEN gene (GenBank 5728) or loss of heterozygosity of the chromosome on which the PTEN gene resides in GBM, likely contributing to the overactivity of this pathway. Temsirolimus and everolimus are both inhibitors of the mammalian target of rapamycin. Temsirolimus as monotherapy was well tolerated but showed little efficacy in recurrent GBM\textsuperscript{18}; combination studies with multiple other agents are ongoing. Perifosine is a direct AKT inhibitor and is also under evaluation in recurrent malignant glioma.

**RAS/RAF/Mitogen-Activated Protein Kinase Kinase/Mitogen-Activated Protein Kinase Pathway.** Another second-messenger system activated by EGFR and PDGFR begins with the RAS protein, which initiates a number of signaling cascades, including that of mitogen-activated protein kinase, which has been implicated in cell proliferation. Farnesyl transferase inhibitors inhibit the enzyme that activates RAS; the most extensively studied in GBM is tipifarnib (R115777), which was well tolerated but showed little efficacy in recurrent GBM\textsuperscript{18}; combination studies with multiple other agents are ongoing.

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**Figure.** Selected growth factor pathways demonstrating targets for new molecular agents, with examples of agents currently under study. EGFR indicates epidermal growth factor receptor; GDP, guanosine diphosphate; Grb2, growth factor receptor-bound 2; GTP, guanosine triphosphate; HGF, hepatocyte growth factor; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; mTOR, mammalian target of rapamycin; P, phosphate group; PDGFR, platelet-derived growth factor receptor; PDK, phosphatidylinositol-dependent kinase; PI3K, phosphoinositide-3-kinase; PIP\textsubscript{3}, phosphatidylinositol 4,5-bisphosphate, PIP\textsubscript{3}, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphate and tensin homologue; and TSC, tuberous sclerosis complex.
Epigenetic Alterations. The importance of epigenetic alterations in tumors is being increasingly appreciated. For example, histone deacetylases, enzymes that play a role in the chromatin structure that organizes DNA and regulates gene transcription, are known to have a role in multiple cancers, including GBM. Vorinostat, a histone deacetylase inhibitor, has shown modest benefit as a single agent in GBM and is currently being tested in combination regimens.

Methods for Local Drug Delivery

One of the major challenges of chemotherapy for GBM is the achievement of adequate drug concentration within the tumor itself. The blood-brain barrier, although often impaired in areas of bulky tumor, still acts as a barrier against many drugs, particularly in the periphery of the tumor, which is often highly infiltrative. Therefore, a variety of alternative delivery methods have been evaluated. One such method is the placement of drug-containing wafers. Carmustine (bischloroethyl nitrosourea) is a nitrosourea compound, and carmustine-impregnated wafers (Gliadel wafers; MGI Pharma, Bloomington, Minnesota) have been placed into the surgical cavity after tumor resection, with modest efficacy. The combination of carmustine-impregnated wafer placement with EBRT has not been formally studied, although it appears well tolerated.

Convection-enhanced delivery is another strategy for local drug delivery. The technique involves the placement of several catheters into a surgical cavity immediately after resection; antineoplastic agents are then delivered through the catheters using convection, which improves distribution within the surrounding tissue where residual tumor cells persist. Early-phase studies using convection-enhanced delivery to deliver cintredekin besudotox (a recombinant protein combining portions of the exotoxin proteins) in patients with recurrent malignant gliomas have been promising, although neither it nor any other agent administered via this technique has received approval yet.

ADVICES IN IMMUNOTHERAPY

Immunotherapeutic treatment for glioma includes active and passive strategies. Active immunotherapy upregulates an immune response to tumor and can confer long-term immunity that potentially continues to provide protection against future tumor recurrence. Passive immunotherapy involves the transfer of immune effectors to achieve an immediate effect but does not generate long-term immunity.

Active Immunotherapy

A variety of strategies are being pursued to induce cytokine secretion directly within tumors because systemic exposure leads to excessive toxic effects. Techniques using direct cell transplants or genetically engineered viral vectors to induce cytokine production within GBM are currently undergoing preclinical evaluation. Another area of active research is that of pattern recognition receptors and their agonists. Double-stranded RNA (dsRNA), a nucleic acid variant normally associated with viruses, is one such agonist. A few clinical trials using poly-ICLC (polynosinic-polycytidylic acid stabilized with polylysine and carboxymethyl cellulose), a dsRNA moiety, have included patients with GBM and have been published, with mixed results.

Tumor vaccines attempt to induce the immune system to generate a response against the tumor. One of the few known truly tumor-specific antigens is EGFRvIII. A peptide vaccine in which the sequence encompasses the mutated segment of EGFRvIII has demonstrated a cytotoxic response against gliomas in preclinical studies. This vaccine, in combination with RT and temozolomide, is being studied in a phase 2/3 trial for patients with newly diagnosed tumors that contain the mutation in question. Several other promising tumor vaccine strategies are also being used in clinical trials.

Dendritic cells are professional antigen-presenting cells that can be primed with tumor antigen ex vivo and then readministered to the patient, where they mediate T-cell activation. Numerous preclinical studies demonstrate that dendritic cells pulsed with glioma antigens can prime a cytotoxic lymphocyte response that is tumor specific; phase 1 and 2 clinical trials have been completed using dendritic cell strategies, with encouraging results.

Passive Immunotherapy

Antibody-mediated drug delivery is a strategy designed with the dual purpose of increasing the local drug concentration while minimizing nonspecific systemic exposure. Monoclonal antibodies targeting glioma-specific structures have been coupled to radionuclides (radioimmunoconjugates), exotoxins (immunotoxins), or chemotherapeutic agents and are administered locally. Antigens that are overexpressed in tumors relative to normal tissue are typically used, such as mutant EGFR, tenascin, and interleukin 4 or interleukin 13 receptors.

ADVANCES IN GENE THERAPY

Gene therapy is based on the insertion or modification of genes into a cell to treat a disease. Gene delivery can be accomplished using a variety of vectors, from viruses to cell-based systems to synthetic vectors. In gliomas, viral vectors have been used to deliver suicide genes, pro-apoptotic genes, p53, cytokines, and caspases. Such studies have shown promising preclinical results, but clinical trials have been limited by the fact that transduced cells were found only within a very short distance of the delivery site. Synthetic vector research has focused on the use of nanoparticles. Liposomal vectors, for example, have been used to deliver therapeutic genes in the preclinical setting.

OTHER THERAPIES

A variety of other novel therapeutic approaches are also currently being researched, including the use of alternating electrical fields to disrupt cell division via a device called NovoTTF-100A (NovoCure Ltd, Haifa, Israel).
currently in phase 3 trials, and the use of thermal lasers to denature tumor tissue.

In conclusion, the survival of patients with GBM continues to improve, albeit more slowly than we would like. A wide variety of new techniques and agents are currently under study, alone and in combination. Increased collective experience in their use and improved understanding of the complex biology of GBM may allow for more rational and effective therapy selection for patients, further extending survival in the years to come.

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