Introduction

Glioblastoma multiforme is the most common glioma arising in adults and accounts for approximately 75% of all newly diagnosed glioma cases in adults. Based on a study by the European Organization for Research and Treatment of Cancer (EORTC) by Stupp et al, which found a 2.5-month improvement in the mean overall survival (OS) for the addition of temozolomide to surgery and adjuvant radiotherapy, the current standard treatment for patients with glioblastoma presenting with a good performance status consists of maximum safe surgical resection followed by adjuvant partial brain radiotherapy with concurrent temozolomide and subsequent continuation of temozolomide for 6 cycles. Even with this approach, however, the mean OS for patients receiving trimodality therapy was only 15 months, with a 9.8% 5-year OS rate.

Patients who benefited most from the addition of temozolomide were those with O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, although nonmethylated patients also derived a statistically insignificant survival benefit. In older patients and in those with a poor performance

Current Status of Immunotherapy and Gene Therapy for High-Grade Gliomas

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Background: Despite improvements in surgical technique, radiation therapy delivery, and options for systemic cytotoxic therapy, the median survival for patients with newly diagnosed glioblastoma multiforme remains poor at 15 months with trimodality therapy. Multiple immunologic approaches are being tested to enhance the response of these tumors to existing therapy and/or to stimulate innate immune responses.

Methods: We review the existing data that support the continued development of immunologic therapy in the treatment armamentarium against glioblastoma multiforme, with a focus on clinical data documenting outcomes.

Results: In phase I and phase II trials, antitumor vaccines (dendritic and formalin-fixed) have demonstrated clinical efficacy with mild toxicity, suggesting that innate immune responses can be amplified and directed against these tumors. Suicide gene therapy (gene-mediated cytotoxic therapy) using a number of viral vectors and molecular pathways has also shown efficacy in completed phase I and ongoing phase II trials. In addition, neural stem cells are being investigated as vectors in this approach.

Conclusions: Although phase III data are needed before immunologic therapies can be widely implemented into clinical practice, the existing phase I and phase II data suggest that these therapies can produce meaningful and sometimes durable responses in patients with glioblastoma multiforme with mild toxicity compared with other existing therapies.
status, hypofractionated radiotherapy without concurrent temozolomide has been shown to be as effective as standard fractionated radiotherapy, and it prolongs survival when compared with supportive care alone.

More recently, single-institution series have demonstrated the safety of hypofractionated radiotherapy with concurrent temozolomide. Ongoing studies by the Radiation Therapy Oncology Group (RTOG) are independently assessing the benefit of adding up-front bevacizumab and cilegitudine to this regimen in patients with a good performance status, but no efficacy results are yet available from these trials. The preliminary results from a study (RTOG 0525), which randomized patients with a good performance status to the previously mentioned regimen with or without dose-intensified temozolomide after concurrent therapy, show no improvement in outcome with dose intensification over standard therapy, although long-term mature data are still pending.

Given these poor outcomes, even in patients with a good performance status treated with trimodality standard therapy, considerable interest has arisen in finding alternate and/or complementary approaches to treating these patients. One such approach has been the development of immunologic therapies, ultimately designed to enhance recognition of these tumors by the patient’s immune system and to increase the activity of tumor-infiltrating lymphocytes (TILs) against them. In this article, we review the initial clinical results of several phase I and phase II trials that have reported clinical outcomes using a number of approaches to immunologic therapy.

**Dendritic Cell Vaccination**

Anticancer vaccines account overall for about 20% of all systemic cancer treatments under development. One approach to glioma vaccination involves the use of dendritic (antigen-presenting) cell vaccines. With this approach, dendritic cells are extracted from the patient and exposed to antigens expressed by the tumor type to be treated. The same “primed” cells are then infused back into the patient and should activate CD8+ (cytotoxic) T cells via tumor-expressed antigen presented to attack the tumor, which is now being recognized by the host’s immune system.

In the context of immunotherapy for gliomas, tumor antigens being utilized include HER2 (ERB-B2), GP100, TRP2, Epha 2, and K13ra. Some emerging evidence has shown that glioma resistance to radiotherapy and chemotherapy may be mediated by a subset of brain tumor stem cells (BTSCs) that express CD133 and nestin, constituting anywhere between 10% and 70% of the total cell population within high-grade gliomas. These BTSCs may be ideal candidates for tumor-directed vaccination, as they do not seem to respond to conventional therapies.

Phase I and phase II studies have demonstrated efficacy and safety for the use of dendritic cell vaccines in the treatment of glioblastoma. In a phase II study, Sampson et al found that the addition of an anti-EGFRvIII dendritic cell tumor vaccine to standard therapy (gross total or near-total surgical resection > 95% followed by fractionated radiotherapy with concurrent temozolomide) in patients demonstrating no evidence of clinical tumor progression after initial therapy and with a performance status of 80% or better increased the median progression-free survival (PFS) from 6.3 months (in a matched control group from MD Anderson Cancer Center) to 14.2 months (vaccination group; hazard ratio [HR], 2.4; P = .013 favoring the vaccination group). The median overall survival (OS) was also improved with vaccination, from 15.0 months (controls) to 26.0 months (vaccination group; HR 5.1; P = .001 favoring the vaccination group). No grade 3 toxicity related to vaccination was noted (maximum toxicity was grade 2, related to injection-site reactions). Eleven patients who developed recurrent tumors after vaccination had pathological tissue from the recurrent tumor available for analysis, and in 82% of these cases, the recurrent tumor had lost EGFRvIII expression. A total of 43% of vaccine-treated patients had serologic evidence of an anti-EGFRvIII humoral response; for these patients, the median OS was 47.7 months compared with 22.8 months for vaccinated patients who did not develop serologic evidence of a humoral response (P = .025 favoring the group with serologic evidence of a humoral response). A total of 18% of vaccine-treated patients developed evidence of a cellular immune response based on delayed-type hypersensitivity (DTH) skin testing, and these patients had not reached a median OS at the time of analysis, compared with 23.1 months for those who did not develop evidence of a cellular immune response.

Overall, 54% of patients in this study demonstrated O-MGMT promoter methylation, a subgroup known to have better survival outcomes with temozolomide-based therapy. When the outcomes of promoter-methylated patients (favorable subgroup) treated without vaccination were compared with those of nonmethylated patients (unfavorable subgroup) treated with vaccination, the vaccinated patients were found to have improved PFS (P = .037) and a trend toward improved OS (P = .062). Although this study was not randomized, it demonstrates that in EGFRvIII-expressing glioblastomas, dendritic cell vaccination can improve PFS and OS with minimal toxicity, outcomes are improved in the unfavorable subset of O-MGMT unmethylated patients, and patients who develop greater evidence of humoral and/or cellular immune responses have improved outcomes with this treatment approach. A similar study (phase II CDX110 trial) has been completed, and the results are pending. The authors also contended that these results support the need to develop future randomized trials testing this approach.

In a separate phase I/II trial (EY-DOH-MD 0910072504) conducted and reported by investiga-
tors in Taiwan, a dendritic cell vaccine directed against immunologically enhanced autologous high-grade glioma cells (World Health Organization [WHO] grades 3 and 4) was found to increase the median OS (525 days for vaccinated patients vs 380 days for historical matched control group) and the 3-year OS (37.5% vs 3.2% favoring vaccinated group) for 16 patients with WHO grade 4 disease. In brief, this technique involved removal and isolation of patient dendritic cells, tumor resection, tumor cell growth in culture with immune enhancement provided by interferon (IFN)-γ and heat-shock treatment, tumor cell exposure to radiation (100 Gy via a cesium source), admixture of dendritic cells and treated tumor cells to generate vaccine, and vaccine administration. For patients who had pathological tumor tissue available for analysis after vaccination (due to re-resection), there was an increase in the total number of CD8+ TILs and a reversal of the prevaccination CD4 to CD8 ratio, suggesting that vaccination did indeed stimulate the development of TILs against these tumors.14

There are several notable differences between this trial3,4 and the trial conducted by Sampson et al.10 First, this trial included both WHO grade 2 and 4 tumors, whereas the Sampson trial evaluated only glioblastomas (WHO grade 4). Second, the vaccine in this trial did not target a specific tumor antigen but instead targeted the patient's autologous glioma cell line raised in culture. Third, the patients treated in this trial did not receive temozolomide therapy, whereas all patients in the Sampson trial were so treated. Fourth, O-MGMT promoter status was not evaluated in this trial, and so the results are not stratified on this basis. Fifth, this trial included both initially treated tumors and recurrent tumors, whereas all patients included in the Sampson trial were treated at initial presentation. Finally, this trial did not identify evidence of humoral or cellular immune responses to vaccination, and the results are therefore not stratified on this basis. Nonetheless, both trials demonstrate the efficacy and overall safety of dendritic cell vaccination against high-grade gliomas and support the conduct of future randomized studies.10,14

This conclusion is supported by a recent open-label nonrandomized study reported by a group from China.16 In this study, 25 patients with newly diagnosed glioblastoma multiforme (aged 20 to 60 years with a performance status of 60% or better) were treated with surgical resection followed by adjuvant concurrent radiotherapy and temozolomide and then 6 cycles of temozolomide, with or without delivery of a dendritic cell vaccine raised against heat shock-enhanced autologous glioblastoma cell lines raised in culture and mixed with isolated patient dendritic cells. With this technique, heat shocking was used to induce apoptosis of glioblastoma cells that had been directly harvested from the patient at the time of resection. Peripheral blood-derived dendritic cells were then utilized to generate the tumor-derived vaccine after plating and culturing in recombinant human granulocyte macrophage colony-stimulating factor (rhGM-CSF), AB serum, and recombinant human interleukin-4 (rhIL-4). These dendritic cells were enriched with the tumor antigen derived from heat shocking, and subsequently, unlike the previous trials, tumor vaccine was administered early in the treatment course, starting on postoperative day 21 and then delivered on days 28, 42, and 56. Immunologic evidence of response to vaccination was assessed by comparing the levels of immunologic cell types (CD3+, CD3+4+, CD4+CD8+, and NK natural killer [NK] cells) and certain cytokines (IL-2, IL-12, and IFN-γ) before and after vaccination. Following vaccination, there was a significant increase in both cellular response and cytokine levels compared with those in nonvaccinated patients ($P < .05$ for both), suggesting induction of immune response in these patients with vaccination. Although there was no difference noted in the tumor control rate at 6 months, there was a significant improvement with vaccination at 9 months ($P < .05$). Vaccination was also noted to improve patients' quality of life (QOL; $P < .05$), median OS, 1-year OS, 2-year OS ($P < .05$), and time to tumor recurrence (11.92 months vs 7.75 months; $P < .05$). No significant increase in toxicity was noted with the addition of vaccination. The real importance of this study is that it demonstrates the safety and efficacy of vaccine administration early rather than later in the course of treatment.

**Autologous Formalin-Fixed Tumor Vaccine**

Limitations to the use of dendritic cell vaccination include the time, expense, and relatively low yield of dendritic cell isolation, culture, and priming. An alternative approach involves the use of formalin-fixed tumor cells, which are obtained at the time of tumor resection; they are fixed, fragmented and centrifuged, mixed with an extract of the BCG vaccine, further mixed with tuberculin microparticles and soluble tuberculin to generate vaccine, and then infused into the patient after performing DTH-2 tests to verify patient response to the BCG vaccine.33-37

Muragaki et al.35 reported the results of a phase I/IIa trial in which 22 patients with glioblastoma were treated with surgical resection followed by postoperative radiotherapy without temozolomide or other chemotherapy. All patients received formalin-fixed tumor vaccine beginning at a radiotherapy dose of 32 Gy to 36 Gy. Three vaccination courses were conducted at weekly intervals, each cycle consisting of 5 intradermal injections. At a median follow-up of 19 months, the median OS was 21.4 months, the median PFS was 7.6 months, and only grade 1 toxicity (injection-site reaction) could be attributed to immunotherapy. A direct correlation was noted between DTH-2 skin reaction and both OS and PFS after vaccination. The median OS and PFS were 22.6 months and 13.9 months, respectively, for patients with a 12-mm or greater DTH-2 reaction compared with...
14.4 months and 4.3 months, respectively, for patients with a < 12-mm DTH-2 response (P = .019 and < .05, respectively), suggesting that immune response to the BCG vaccine correlated with clinical response to formalin-fixed tumor vaccine.

A smaller corollary study by the same group consisted of 3 patients with either initial or recurrent glioblastoma multiforme who were treated with standard therapy (surgery followed by radiotherapy and/or temozolomide) followed by concurrent formalin-fixed tumor vaccine and temozolomide therapy during the first cycle of adjuvant temozolomide. All patients then went on to receive 5 additional cycles of temozolomide alone. Minimal toxicity was noted with the addition of tumor vaccine to temozolomide. Two patients received additional surgical therapy, and in both cases, an influx of CD3+CD8+ TILs and a decrease in the MIB-1 labeling index score (proliferative marker) were noted. Partial radiographic responses were also seen in 2 patients. The authors concluded that the combination of formalin-fixed tumor vaccine and concurrent temozolomide was safe and warranted further testing.

**Gene-Mediated Cytotoxic Therapy**

A different approach to immunotherapy for high-grade gliomas involves the direct inoculation of engineered viruses into the resection bed at the time of surgery. This technique, also known as suicide gene therapy, generally involves the introduction of an enzyme into a tumor, which converts a nontoxic prodrug into a lethal byproduct. For example, genetically engineered viruses containing the gene-encoding herpes simplex virus thymidine kinase (HSV-tk) are designed to be taken up preferentially by residual tumor cells in and around the resection cavity as well as endothelial cells within the tumor vasculature. Following their uptake, the patient is given an antiviral prodrug, and this drug is converted by HSV-tk into a cytotoxic nucleotide analog, which has antiangiogenic and direct antitumor cytotoxic effects as well as a strong immunostimulatory effect.

In a germinal National Institutes of Health (NIH) study by Culver et al, rat cerebral gliomas were injected stereotactically with murine-derived fibroblasts containing a retroviral vector expressing HSV-tk and were subsequently treated with ganciclovir, causing macroscopic and pathological complete tumor regression. One adenovirus vector engineered to contain HSV-tk has shown activity against recurrent malignant gliomas in a pilot trial, with acceptable toxicity noted with dose levels of 2 × 10^11 vector particles.

A subsequent phase I/II trial, which enrolled 12 patients with newly diagnosed WHO grades 3 and 4 gliomas, has been completed. These patients underwent maximum safe tumor resection; at the time of resection, 100 μL of HSV-tk was injected to a depth of 1 cm to 2 cm at up to 10 sites around the resection cavity. Valacyclovir 2,000 mg was given 3 times daily for 14 days starting on postoperative days 1 to 3. Standard fractionated radiotherapy was given starting by postoperative day 9, and temozolomide was initiated after valacyclovir administration was completed. Toxicity directly related to HSV-tk was generally mild (grade 1 or 2), with 1 patient developing a grade 3 wound complication, 1 patient developing grade 3 hyponatremia, and 1 patient developing grade 3 elevation in aspartate aminotransferase levels. No grade 4 or 5 immunotherapy-related toxicity was noted. The median PFS was 9.1 months, whereas the median OS was 12.4 months, and the 1- and 2-year OS rates were 33% and 25%, respectively. No correlation was noted between O-MGMT promoter methylation status and outcomes, but the reason for that may be the result of the small study size. Pseudoprogression was noted on imaging in 3 of 12 patients and subsequently resolved in all cases. All patients had stable or improved QOL, which was determined using the Functional Assessment of Cancer Therapy-Brain (FACT-Br) questionnaire. Subsequent tumor resection occurred in 4 patients, and in all of these cases, a significant CD3+CD8+ T-cell infiltrate and a CD8+ macrophage infiltrate were noted at anywhere from 7 months to 22 months after HSV-tk administration, suggesting durable immunostimulation. According to the authors, the significant activity noted with HSV-tk was due to a high rate of transduction, whereas prior trials utilizing a retroviral vector were unsuccessful because of poor transduction.

Another small study compared outcomes for patients treated with adenoviral-tk, retroviral-tk, and adenoviral marker vectors and found that survival was approximately doubled with the adenoviral-tk vector. A prior phase II trial utilizing a similar adenoviral vector to treat both initial and recurrent high-grade gliomas was reported by Sandmair et al. It showed a significant improvement in survival outcomes compared with those of a historical control group, but the subsequent randomized phase II trials showed only a trend toward better outcomes over standard therapy (no statistically significant difference). Thus, the overall clinical results of gene-mediated immunotherapy for high-grade gliomas have been mixed.

A novel approach to suicide gene therapy involves the use of neural stem cells (NSCs) as a vector. Located in the subgranular zone of the hippocampus and the subventricular zone adjacent to the lateral ventricles, NSCs are pluripotent cells capable of proliferating throughout life (including adulthood) and differentiating along neuronal or glial cell lines. They also contain migratory capability and can home to areas of damage to reconstitute the injured cellular elements in that area. There is also some evidence that they are involved in learning activities via the generation of new neural pathways. In addition, it has been demonstrated that immortalized human NSC clones can home to areas of tumor infiltration. In this capacity, they can be used to enhance the local...
immune response to the tumor and/or serve as a vector for the delivery of either suicide genes (enzymes, as above) or immunostimulating cytokines such as IL-4, IL-12, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). \textsuperscript{32} Studies performed at Children's Hospital of Boston demonstrated that when injected into adult rodent gliomas directly, or after intravenous injection or implantation elsewhere in the brain, NSCs would home to the tumor and distribute themselves widely throughout the lesion. Those same cells also demonstrated the ability to deliver cytosine deaminase to these tumors, causing subsequent tumor regression. \textsuperscript{32}

In the context of immunotherapy for gliomas, NSCs may be injected intracranially or intravenously and have been shown to home to tumor foci under the influence of a number of chemotactic signals and growth factors, which are secreted by gliomas and their surrounding stromal elements. These factors include stem cell factor-1 (SCF-1), monocyte chemoattractant protein-1 (MCP-1), stromal cell-derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR), and hypoxia (via a number of mediators). There is some evidence that these pathways converge on the PI3K pathway, as one recent study showed that inhibition of this pathway led to a loss of NSC homing under the influence of a number of signals. \textsuperscript{53} Genes that have been effectively introduced into gliomas using NSCs as a vector include cytosine deaminase, which converts the prodrug 5-fluorocytosine (5-FC) intracellularly into the toxic product 5-fluorouracil (5-FU), and HSV-tk, as described previously. NSCs have also shown the ability to deliver PEX (a matrix metalloproteinase 2 inhibitor) and thereby inhibit the formation of new tumor vasculature (reduce angiogenesis). \textsuperscript{53}

At present, the use of tumor-tropic NSCs in the treatment of gliomas has been limited to animal studies, but the NIH has now approved City of Hope Hospital in Duarte, California, to conduct a clinical pilot trial involving 10 patients. \textsuperscript{54} The trial will utilize immortalized human NSCs bearing cytosine deaminase in the treatment of recurrent high-grade gliomas. Following the administration of NSCs, patients will be treated with 5-FC for 7 days, starting on day 5. Thus far, there are no other reported clinical trials utilizing NSC vectors for the treatment of high-grade gliomas in humans.

Finally, there is also some evidence that marrow-derived stem cells may have the ability to deliver targeted therapy when directly inserted into tumors, with subsequent reduction in tumor growth after delivery. Studeny et al. \textsuperscript{39} demonstrated that such cells, when directly inoculated into tumors, could effectively generate high levels of IFN-β within these tumors and inhibit growth in vivo.

Conclusions

Although phase III data are lacking, the existing preclinical, phase I, and phase II data suggest that immunotherapy potentially offers a new approach in the treatment of high-grade gliomas, both in the initial treatment setting and in the context of recurrent tumors. These approaches are associated with mild toxicity when compared with other available treatment options, and given the overall poor prognosis in this disease, further studies are warranted. \textsuperscript{56} No one approach to immunotherapy in this treatment setting has shown clearly superior outcomes, and to date no studies have been performed to compare clinical outcomes among different techniques.

References


