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Review

Topics in Chemotherapy, Molecular-targeted Therapy, and Immunotherapy for Newly-diagnosed Glioblastoma Multiforme

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Abstract. *Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, and it is associated with poor survival. The standard therapy for newly-diagnosed GBM is radiotherapy with concurrent temozolomide following maximal surgical resection. To improve the outcome of these patients, combinations of the standard therapy plus molecular-targeted agents have been tested in clinical trials. However, the addition of gefitinib to the standard therapy did not appear to improve clinical outcome, and the standard therapy plus bevacizumab showed no improvement in overall survival, although a 4-month improvement in progression-free survival (PFS) was observed. Phase II data have indicated the potential efficacy of talampanel combined with the standard therapy for patients with newly-diagnosed GBM, and these findings are awaiting validation in phase III trials. In addition, phase II trials have demonstrated that adjuvant immunotherapy is effective and tolerable for treatment of patients with GBM. In this article, we discuss topics in chemotherapy, molecular-targeted therapy, and immunotherapy for patients with newly-diagnosed GBM.*

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, accounting for approximately 70% of high-grade gliomas (1). Despite recent advances in chemotherapy, radiotherapy (RT), and surgical resection, the prognosis of patients with GBM is poor. Pre-treatment patient characteristics, such as age at diagnosis and Karnofsky performance status, are the best predictors of survival (2). The standard treatment for patients with GBM consists of maximal surgical resection followed by adjuvant RT. At present, the standard-care for postoperative patients with newly-diagnosed GBM is 60 Gy in 30-33 fractions with concurrent temozolomide (3). Although the addition of temozolomide prolongs the survival of such patients, the median survival time (MST) is only 14.6 months.

To further improve overall survival, dose escalation to the target volume by innovations in RT is currently being tested (4). This dose-escalation strategy has been reported to reduce the rate of local recurrence. Thus, it seems that RT doses higher than 60 Gy are necessary within an irradiated field to control GBM. However, this strategy may be associated with limitations, including severe toxicity, such as radiation-induced necrosis. Furthermore, dissemination to the meninges or spinal cord has frequently been reported in patients with GBM (5). Therefore, chemotherapy and molecular-targeted agents are also being tested to improve the clinical outcomes of patients with GBM. Furthermore, immunotherapies, which are emerging as the newest cancer treatments, have also demonstrated feasibility and potential efficacy in these patients (6). In the present article, we review the history of chemotherapy, the current outcomes of clinical trials using combined treatments with molecular-targeted agents and RT, and the outcomes of trials using immunotherapy for patients with newly-diagnosed GBM.

Chemotherapy for GBM

Historical context of chemotherapy. GBM is considered to be resistant to chemotherapy. There are two main reasons that have been established for this resistance (7); the first is the presence of the blood–brain barrier (BBB), which blocks the

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transport of most molecules larger than 500 Da. Therefore, the BBB is an obstacle to the adequate delivery of chemotherapy agents to brain tumors, particularly the infiltrating component of the tumors, where the malignant cells have intercalated into normal brain parenchyma (8). Only small and lipophilic molecules are able to cross the BBB to reach their target. The second reason for the chemotherapy resistance of GBM is the intrinsic resistance of the GBM cells, particularly glioma stem cells (9), which show enriched activity of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) (9) and have demonstrated resistance to alkylating agents (10). Considering these characteristics of GBM, strategies have been developed and evaluated combining chemotherapy with RT.

Historically, intravenous injection of nitrosoureas, which are lipid-soluble agents that cross the BBB, has been considered to have potential for improving the outcomes of patients with GBM receiving RT (11). A meta-analysis by Stewart *et al.*, in which more than 3,000 patients with malignant glioma were included and the bulk of the trials utilized intravenous injection of nitrosoureas, demonstrated a modest increase in MST due to the addition of chemotherapy to RT (12). However, only a 5% increase in survival at two years (from 15% to 20%) was observed using this combination of chemotherapy and RT. In addition, in this meta-analysis, 37% of patients had prognostically favorable, lower-grade gliomas (13). There was no evidence that the effect of intravenous injection of nitrosoureas differed in any group of patients according to age, gender, performance status, or extent of surgical resection. These results drove researchers to explore the use of novel chemotherapy agents in combination with RT.

Temozolomide. At present, temozolomide is the standard agent used for concomitant and adjuvant chemotherapy with RT to treat patients with GBM. Temozolomide is an imidazotetrazine derivative synthesized in a pathway that produces imidazo-1,2,3,5-tetrazines (13). Temozolomide, which is an oral alkylating agent, is rapidly absorbed, with maximum plasma concentrations observed at 30 to 90 min after oral intake. Its plasma half-life is approximately 2 h (14). With regard to pharmacokinetics, the area under the concentration–time curve (AUC) for temozolomide in cerebrospinal fluid corresponds to approximately 20% of the plasma AUC (15). Temozolomide methylates the O^6-position of guanine, which leads to cell-cycle arrest in the radiosensitive G_2/M phase (16). It is also known that temozolomide can suppress ionizing radiation-induced migration and invasion in glioma cells (17).

In the clinical setting, the efficacy of combined temozolomide and RT in patients with GBM has been shown in a series of studies performed by Stupp *et al.* (3, 18, 19). In a randomized phase III trial including 573 patients with GBM, the European Organization for Research and Treatment of Cancer (EORTC) 26981-22981/National Cancer Institute of Canada Clinical Trials Group CE3 (NCIC) established temozolomide as the standard chemotherapeutic agent for the treatment of GBM (3). An improvement in MST from 12.1 to 14.6 months was observed due to addition of temozolomide to RT. A survival benefit was observed for all patients, regardless of performance status or age. These results confirmed that the addition of temozolomide to RT is an effective standard therapy for GBM. To date, the standard treatment of patients with GBM is still considered to be postoperative chemoradiotherapy (CRT) with temozolomide followed by six cycles of adjuvant temozolomide.

Carmustine wafers. A carmustine wafer is a form of medication of carmustine that can be placed and left in the cavity after surgery for GBM, acting as a source of local chemotherapy delivery. This wafer circumvents the challenges imposed by the BBB. Recently, Affronti *et al.* showed the effects of carmustine wafers on the survival of patients with newly diagnosed GBM treated with concurrent temozolomide plus RT plus rotational chemotherapy (20). The overall 1- and 2-year survival times, and MSTs for the non-carmustine wafer cohort *versus* the carmustine wafer cohort were 69% *versus* 81%, 29% *versus* 47%, 72.7 weeks and 89.5 weeks, respectively. Although this study was retrospective, the proportion of patients in the carmustine wafer cohort who lived longer than predicted based upon the results of the Stupp regimen was significantly increased. By contrast, Bock *et al.* reported that the combination of carmustine wafers and concomitant CRT should be carefully considered due to its significant toxicity (21). In their study of 44 patients with newly diagnosed GBM who received the carmustine wafer-supplemented regimen, 19 (43%) experienced grade 3 or 4 adverse events, including hematotoxic events. This percentage was much higher than that previously reported by Stupp *et al.* (3). Therefore, prospective trials are required to rigorously compare the carmustine wafer-supplemented regimen to the Stupp regimen.

Molecular-targeted Agents for GBM Treatment

Epidermal growth factor receptor (EGFR) inhibitors. Several molecular-targeted therapy agents have been tested in patients with newly-diagnosed GBM. EGFR is a tyrosine kinase receptor that plays important roles in cell survival, proliferation, migration, and differentiation in many types of cancers (22). Pre-clinical data have suggested that overexpression of EGFR confers radiation resistance in malignant glioma and that the antagonism of EGFR restores radiosensitivity (23). Therefore, the clinical efficacies of small-molecule tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, have been investigated. Mellinghoff *et al.* critically reviewed a small subset of patients with

recurrent malignant glioma enrolled in three multi-Institutional clinical trials who achieved a partial or minor response after treatment with gefitinib or erlotinib (24). However, RTOG 0211, which was a single-arm phase I/II study, showed that the addition of gefitinib to RT did not improve outcome in a general population of patients with newly-diagnosed GBM. EGFR expression analysis was not of prognostic value in patients treated with RT and concurrent and adjuvant gefitinib (25). Taken together, further evaluation of the combined use of RT, temozolomide, and TKIs is warranted in a larger prospective trial, including additional studies to elucidate the underlying functional mechanisms at the molecular level.

Vascular endothelial growth factor (VEGF) inhibitors. GBMs are highly vascularized tumors that rely on angiogenesis and secrete an abundance of VEGF (26). Bevacizumab, which is a humanized monoclonal antibody that targets VEGF, has shown potential antitumor effects in patients with GBM. In a phase II trial performed by Lai *et al*., 70 patients were treated with bevacizumab, temozolomide, and concurrent RT of 60 Gy in 30 fractions after surgery, followed by maintenance bevacizumab and temozolomide after the completion of concurrent therapy for 24 months or until disease progression, at which time bevacizumab alone was continued (27). Compared to historical controls, the addition of bevacizumab resulted in improved PFS but no significant change in MST; the PFS and MST were 13.6 and 19.6 months, respectively, compared to the EORTC/NCIC trial cohort (3, 18). Toxicity attributable to RT/temozolomide was similar to that observed in the EORTC/NCIC trial, and additional toxicities were consistent with those reported in previous bevacizumab trials (28). To determine whether the addition of bevacizumab to temozolomide and RT improves treatment efficacy, a multi-Institutional randomized phase III trial (RTOG 0825) was performed (29), in which a total of 978 patients were registered, and 637 underwent randomization. Although the median PFS was longer in the bevacizumab group than the placebo group (10.7 months *versus* 7.3 months), there was no significant difference in the duration of overall survival between these two groups (medians of 15.7 and 16.1 months, respectively). Furthermore, higher rates of neurocognitive decline, increased symptom severity, and a decline in health-related quality of life were observed over time in patients treated with bevacizumab. The AVAglio trial, which was very similar in design to RTOG 0825 (including provisions for crossover) and involved 921 patients, also showed an improvement in PFS, but there was no significant difference in the duration of overall survival for the bevacizumab group (30). Considering the slight benefit on PFS and its higher toxicity rates, the addition of bevacizumab to combined CRT and temozolomide treatment does not seem to represent an effective therapy for patients with newly-diagnosed GBM.

Antagonists of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor. Glutamate is a major excitatory neurotransmitter in the central nervous system. Glioma cells release glutamate in concentrations that are toxic to surrounding neurons and to glia (31). Recent studies have suggested that the glutamatergic system also plays a key role in the proliferation, survival, and migration of gliomas, perhaps by the activation of the Akt pathway (32-34). Talampanel is an oral, non-competitive antagonist of the AMPA subtype of glutamate excitatory amino acid receptors, with excellent brain penetration (35). A phase II trial designed to estimate overall survival in patients with newly-diagnosed GBM treated with talampanel in addition to standard RT and temozolomide reported an MST of 18.3 months. This study also showed that talampanel was well-tolerated and did not increase the known hematological or non-hematological toxicities of temozolomide. Most notably, fewer patients in this talampanel study had methylated *MGMT* compared to the EORTC study (29% *versus* 43%, respectively). Despite these findings, the overall survival of comparable patients receiving talampanel was superior (2-year survival of 42% *versus* 27%, respectively). A randomized, placebo-controlled trial is needed to rigorously assess the value of this promising therapeutic approach.

Immunotherapies for GBM

Antitumor vaccines. Recent studies have indicated that antitumor immunotherapy for GBM may be effective and well-tolerated in clinical trials. Antitumor vaccines represent the most established and well-studied immunotherapeutic modality. Several known tumor-associated antigens are currently being targeted in patients with GBM, including HER2, TRP2, GP100, MAGE1, IL13a2, and AIM2 (36). The most extensively studied vaccine strategies have targeted the tumor-restricted neo-antigen EGFR variant III (EGFRvIII) (37). EGFRvIII is a constitutively activated and immunogenic mutation that is not expressed in normal tissues but is widely expressed in GBM (38). For patients with GBM who survive for a year or longer after diagnosis, the expression of EGFRvIII is an independent negative prognostic indicator of survival (39). Thus, EGFRvIII is thought to be an ideal target for antitumor immunotherapy.

A phase II multi-center trial was undertaken to assess the immunogenicity and efficacy of an EGFRvIII-targeted peptide vaccine called PEPvIII (40) (which is also known as rindopepimut or CDX-110). In this trial, adults with newly-diagnosed EGFRvIII-expressing GBM with gross total resection (>95%) and a Karnofsky performance status of ≥80% who had no evidence of progression after standard concurrent CRT using temozolomide were eligible for vaccination. No significant adverse effects were reported,

and the MST among this cohort was 26.0 months. At recurrence, 82% of patients had lost EGFRvIII expression. Although the effects of RT and chemotherapy on EGFRvIII have not yet been fully-characterized (41), EGFRvIII-targeted vaccination in patients with GBM is a promising strategy. A phase III randomized trial to investigate the efficacy of the addition of the EGFRvIII-targeted peptide to the current standard of care in patients with newly diagnosed GBM is ongoing (NCT01480479).

The Wilms' tumor 1 (WT1) peptide vaccine has also been tested in patients with GBM. The *WT1* gene was identified as a gene responsible for Wilms' tumor. It encodes a zinc finger transcription factor that is involved in cell proliferation and differentiation, apoptosis, and organ development (42). The wild-type *WT1* gene was shown to be overexpressed in various types of solid tumors. Thus, the WT1 protein was found to be an attractive target antigen for immunotherapy to treat these malignancies (43). Izumoto *et al*. reported the results of a phase II trial of WT1 peptide vaccination for 21 patients with WT1/human leukocyte antigen (HLA)-A*2402–positive recurrent GBM (44). The protocol was well-tolerated, and only local erythema occurred at the WT1 vaccine injection site. The disease control rate (cases with complete or partial response as well as those in which disease was stable) was 57.1%. The median PFS was 20.0 weeks, and the 6-month PFS was 33.3%. WT1 vaccine therapy may represent a promising treatment strategy for patients with WT1/HLA-A*2402-positive newly-diagnosed GBM. Further clinical studies of WT1 vaccine therapy or the addition of WT1 to the combination CRT/temozolomide treatment in patients with newly diagnosed GBM are warranted.

Immune cell therapy. To enhance the therapeutic response of malignant gliomas, an adoptive cell transfer therapy using *ex vivo*-activated autologous lymphocytes has been considered to be a promising approach. Autologous lymphokine-activated killer (LAK) cells have been administered to patients with glioma in clinical studies with some positive results (45, 46). However, LAK cell therapy alone is not considered very efficient for clinical use because of its low killing activity, non-specificity, and the side-effects associated with simultaneous IL2 administration (45, 46).

Administration of tumor antigen-loaded dendritic cells (DCs) has the theoretical advantage of activating host lymphocytes while bypassing the requirement for antigen processing by host antigen-presenting cells, which are often dysfunctional in patients with cancer (47). In comparison with LAK therapy, DC administration may be a good strategy from the tumor specificity point of view. Phase I and II studies have demonstrated the efficacy and safety for the use of DC vaccines for the treatment of GBM (48, 49). Ardon *et al*. reported the results of an HGG-2006 phase I/II, single-arm trial (50). This study evaluated the clinical efficacy of the full integration of DC-based tumor vaccination into standard postoperative CRT in 77 patients with newly-diagnosed GBM. Tumor cell lysates and autologous DCs were administered every four weeks after a 6-week course of concomitant CRT. The results showed tolerable toxicity, and the MST was 18.3 months, which compares favorably with the survival data reported by Stupp *et al*., reporting a median OS of 14.6 months (3). These results were used to develop the currently running phase II randomized clinical trial (NCT01280552). However, the immune profiles in the peripheral lymphocyte or delayed-type hypersensitivity skin tests did not reveal any correlation with clinical outcome in the previous studies. A possible explanation for this lack of correlation between the immunological and clinical responses might be that the peripheral immune status does not mirror the immune response that occurs in the tumor itself. Establishing immunological predictors of clinical outcome appears to be necessary to enhance treatment strategies involving the use of DCs.

Immune checkpoint inhibitors. The discovery of immune checkpoints and the development of agents targeting these pathways have led to a novel strategy in cancer therapy. Cytotoxic T lymphocyte antigen-4-blocking antibodies, programmed cell death-1 (PD1)-blocking antibodies, and PD1 ligand antibodies have shown promising results in the treatments of several types of tumors (51-53). These immune checkpoint molecules have not yet been used for the treatment of patients with GBM in the clinical setting. Zeng *et al*. reported that synergistic responses could be achieved using a combination of an antibody to PD1 and RT in a mouse model of glioma (54). RT causes the release of multiple tumor-associated antigens, acting as a vaccine and leading to the generation of immunological memory. Therefore, it is quite reasonable to administer immunotherapy in combination with RT. Zeng *et al*.'s study provided pre-clinical evidence to support the combination of immunotherapy and RT for the treatment of patients with GBM. Further studies are required to evaluate the efficacy of these immune checkpoint agents in the clinical setting.

Conclusion

Despite the existence of state-of-the-art oncological therapies, the prognosis of patients with GBM remains poor. Although various phase II and III clinical trials have been performed, the standard treatment of patients with GBM is still considered to be postoperative CRT with temozolomide followed by six cycles of adjuvant temozolomide. Bevacizumab was expected to be the single most important therapeutic agent for GBM treatment besides temozolomide, and a multi-center international trial showed a significant, 4-month, improvement in PFS using bevacizumab. However,

there was no difference in overall survival among the patients treated with the standard therapy plus either bevacizumab or a placebo.

Several clinical trials are ongoing with the goal of improving the outcomes of patients with newly diagnosed GBM. Among these trials, the phase II data have indicated that immunotherapy may be a new approach for the treatment of GBM, although phase III data are still lacking. Further studies to increase our understanding over the interplay between the immune response in the central nervous system and GBM are warranted to establish the optimal immunotherapeutic approach for the treatment of these patients.

Conflicts of Interest

The Authors declare no conflicts of interest associated with the present study.

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