Temozolomide and Other Potential Agents for the Treatment of Glioblastoma Multiforme

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KEYWORDS

• Temozolomide • Glioblastoma multiforme • BCNU wafers • Bevacizumab • NovoTTF-100A

Key Points

- Glioblastoma multiforme is the most common primary central nervous system tumor, representing approximately 60% of all primary brain tumors in the United States
- Temozolomide has quickly become a part of the standard of care for the modern treatment of stage IV glioblastoma multiforme
- Despite its improvements from previous therapies, median survival remains dismal
- Epigenetic modulation of the MGMT promoter gene by hypermethylation results in decreased MGMT mRNA expression and increased response to temozolomide therapy
- Given the substantial population of patients with resistance to temozolomide treatment, the development of supplemental, combination, or alternative therapies is critical to optimize glioblastoma multiforme management
- Other Food and Drug Adminisration–approved therapies for glioblastoma include BCNU wafers, bevacizumab, and NovoTTF-100A

Glioblastoma multiforme (GBM) is the most common primary central nervous system tumor, representing approximately 60% of all primary brain tumors in the United States and characterized by aggressive invasion throughout adjacent parenchyma.[1] Although untreated patients generally do not survive beyond 3 months, even with optimal patient management median overall survival (OS) is approximately 15 months, with a 2-year survival rate of 8% to 26%.[1–5] Standard of care remains surgical resection with concomitant daily temozolomide (TMZ) and radiotherapy, followed by

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adjuvant TMZ.[1] Given its tendency for rapid tumor growth, GBM is often diagnosed at a point when severe damage to eloquent structures has already occurred. Furthermore, expansion along the white matter tracts and across the corpus callosum is not uncommon, making gross total resection difficult and recurrence nearly inevitable.[2] Given that no standard of treatment has yet been established for recurrent tumors, many clinicians rely on TMZ retreatment with either standard or alternative dosing strategies. Although several studies have not reported any significant benefit from alternative dosing after GBM recurrence, numerous investigations are currently evaluating outcomes to optimize TMZ dosing in first- and second-line settings.[1]

Nevertheless, gross total resection remains the treatment goal (**Fig. 1**), with several studies demonstrating extent of removal being prognostic for OS.[3,4,6,7] It has been reported that with greater than 98% tumor resection, median survival time is 13 months, compared with 8.8 months when less is removed.[3] One recent analysis examining the role of extent of resection concluded that the added benefit of total versus subtotal resection confers at least 3 months of increased survival for patients.[4] However, if lesions are situated in eloquent areas, such as those involved in speech and language comprehension, adjuvant and less caustic therapies may be preferred in place of aggressive surgical resection.

Other prognostic factors have included patient age, apparent tumor necrosis, presence of edema, and Karnofsky Performance Status scores. Despite recent advancements in image-guided surgical resections[8] and the use of fluorescent 5-aminolevulinic acid,[9] surgery does not always translate to improved outcome, most notably in elderly patients with poor presurgical evaluation who present with necrosis, edema, and low Karnofsky Performance Status scores.[2,3,10,11] In a recent report focusing on patients with advanced age, median survival was 8.6 months with greater than 98% resection, and 7.8 months with less than 98% resection. This difference did not reach significance ($P = .13$),[3] indicating that there was no survival advantage appreciated for a gross total resection in older individuals. These patient populations with poor surgical outcomes remain in dire need of effective adjuvant therapy.

TMZ is a chemotherapeutic agent for treatment of glioblastoma,[11] considered standard of care since 2005. TMZ was first approved by the Food and Drug Administration (FDA) for use in recurrent GBM based on the phase II trial by Yung and colleagues[12,13] in which they demonstrated improved 6-month progression-free survival (PFS) and 6-month OS over procarbazine in 225 patients. In the pivotal phase III study, Stupp and colleagues[14] randomized 573 patients from 85 centers for treatment of newly diagnosed GBM with either radiation therapy alone or radiotherapy plus continuous daily TMZ. Their investigation demonstrated an improved 14.6-month median survival in the TMZ group, versus 12.1 months in the control group. Two-year survival was also increased to 26.5% compared with 10.4% for those treated with radiotherapy alone. Regarding long-term outcome, 5-year survival for groups receiving TMZ or radiation therapy alone was 9.8% and 1.9%, respectively.[14–18] Here, we discuss the advantages and limitations of TMZ as a treatment for glioblastoma, and explore possible directions for future research and therapeutic options.

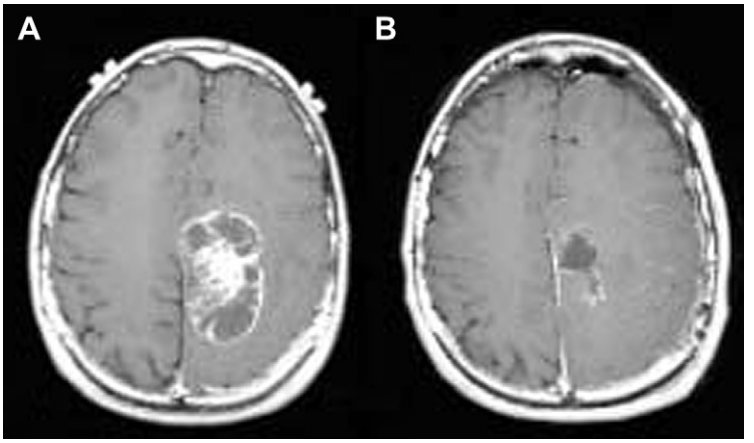

Fig. 1. Gross total resection of a glioblastoma as demonstrated by the (*A*) preoperative and (*B*) postoperative contrast-enhanced axial MRI. (*From* Hentschel SJ, Sawaya R. Optimizing outcomes with maximal surgical resection of malignant gliomas. Cancer Control 2003;10(2):109–14; with permission.)

MECHANISM OF ACTION AND PHARMACOKINETICS

TMZ is a second-generation DNA alkylating agent that disrupts malignant growth and cell cycle repair by methylating the N^7 and O^6 positions of guanine and the N^3 site on adenine 70%, 5%, and 9% of the time, respectively.[19,20] The O^6 position of guanine, although least frequently the target of TMZ, is of unique importance because its methylation results in its pairing with thymine rather than cytosine during DNA replication.[21] This pharmacologically induced injury results in crosslinking of double-stranded DNA, rendering mismatch mechanisms unable to repair the glioblastoma's damaged DNA. A series of double-stranded breaks, calcium-dependent apoptosis, and autophagy after this mismatch ultimately result in cell death.[21–23]

TMZ is an imidazotetrazine derivative prodrug that spontaneously decomposes to 5-(3-methyltriazen-1-yl)imidazole-4carboxamide (MTIC) on entering a basic environment (**Fig. 2**). Because activation of the prodrug does not require first-pass metabolism, hepatic and renal function are not a factor for its performance.[24] Because of TMZ's stability in acidic environments (pH <4), it remains in a prodrug state while passing through the digestive system[25] and is conveniently administered orally.[26] Oral TMZ is rapidly and almost entirely (>99%) bioavailable, and physiologic conditions immediately lead to nonenzymatic decomposition of TMZ into MTIC. MTIC in turn decomposes into 4-amino-5-imidazole-carboxamide (AIC)[27,28] in acidic environments.[26,29–33]

By oral administration, mean maximum peak is at 1.2 hours and mean half-life is 1.9 hours.[29] For patients who have absorption difficulties, nausea, or are too young to swallow TMZ in pill form, intravenous TMZ is an alternative[34] that is biologically equivalent to oral TMZ, with nearly identical pharmacokinetic parameters (half-life, time of maximum peak, and clearance).[35] Because MTIC can be hydrolyzed to AIC in any tissue with subsequent clearance, TMZ does not accumulate in plasma after the standard 5-day therapy cycle. However, some AIC may remain in the body to be used as an intermediate in purine synthesis.[27–30]

Although the blood–brain barrier may be an obstacle for many therapeutic agents, TMZ is a lipophilic molecule that effectively penetrates to glioma cells.[30,36] Additionally, the blood–brain barrier surrounding these tumors is often

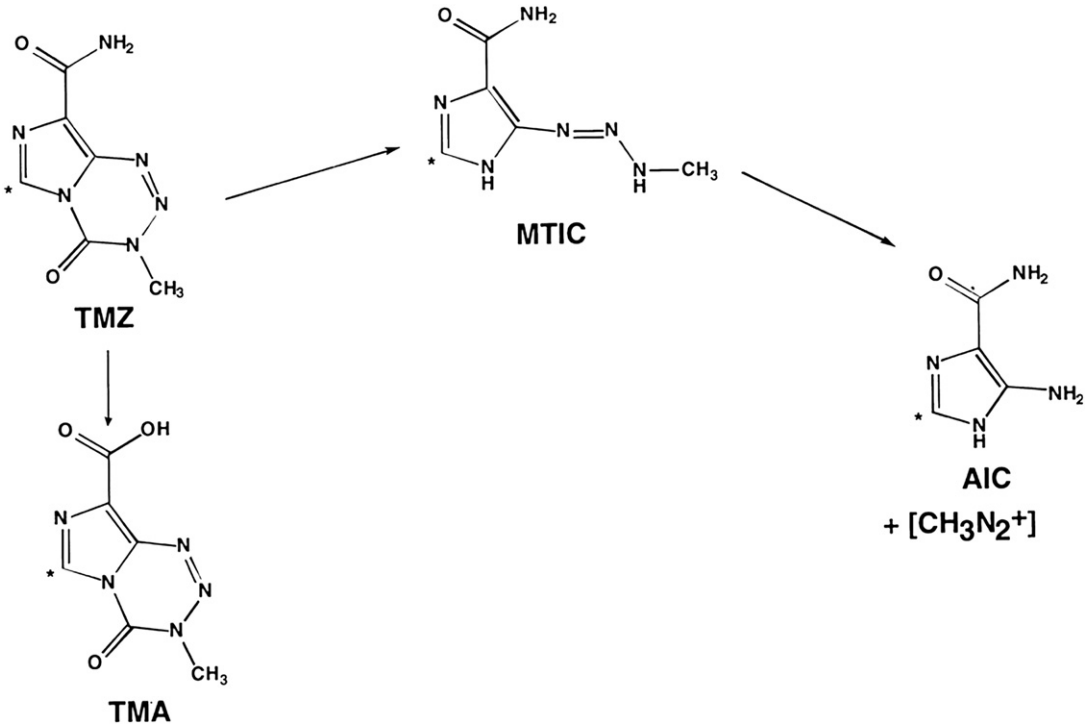

Fig. 2. Metabolic and degradation pathways of temozolomide. AIC, 4-amino-5-imidazole-carboxamide; TMA, 3-methyl-2,3-dihydro-4-oxoimidazo-[5,1-d]-tetrazine-8-carboxylic acid. (*From* Baker SD, Wirth M, Statkevich P, et al. Absorption, metabolism, and excretion of 14C-temozolomide after oral administration to patients with advanced cancer. Clin Cancer Res 1999;5(2):309–17; with permission.)

compromised, and the highly angiogenic characteristics of glioblastoma allow TMZ concentrations in malignant areas to reach higher levels than in normal surrounding tissues.[37] However, it has been suggested that GBM cells with invasion into healthy parenchyma may be exposed to decreased concentrations of TMZ compared with those in closer proximity to disruptions in the blood–brain barrier.[38]

Extensive research is currently underway to elucidate other mechanisms and cascades of TMZ toxicity on glioblastoma cells. A study into the precise changes in gene transcription and protein translation after TMZ treatment identified 1886 proteins that were expressed at greater than twofold differences.[39] Two proteins of particular interest were hypoxia-inducible factor (HIF)-1 (increased in two distinct glioblastoma cell lines between twofold and sixfold) and vascular endothelial growth factor (VEGF) (increased by twofold to fourfold). Although these specific proteins were not increased to the greatest extent compared with several others, they are implicated in control processes that are thought to promote tumorigenesis, despite the fact that TMZ has an overall antitumor effect. The stress of TMZ induces a hypoxic-like state that promotes maintenance of the regulator protein HIF-1α. In turn, HIF-1α becomes concentrated within the cell, with subsequent promotion of angiogenesis, glycolysis, cell cycle maintenance, erythropoiesis, and apoptosis.[39] Because these processes enhance tumorigenesis and autophagy,[40] authors of the study suggest the possibility of a common pathway to the activation of tumorigenesis and tumor destruction,[39] requiring further elucidation on the interplay between these seemingly opposing processes.

Furthermore, TMZ therapy may be able to activate p53 and $p21^{WAF1/Cip1}$-mediated G_2/M arrest with subsequent apoptosis or senescence. However, this chemotherapeutic approach in GBM cells with decreased p53 expression may have a more limited role.[38] This notion has been supported by one study demonstrating improved PFS in patients with p53 overexpression.[41]

SIDE EFFECTS AND QUALITY OF LIFE

Before the approval of TMZ, the standard of care for GBM treatment was surgical resection, followed by radiotherapy and adjuvant nitrosoureas.[14,42,43] Such agents included carmustine or the trio combination of procarbazine (an alkylating agent), lomustine (an alkylating nitrosourea), and vincristine (a mitotic inhibitor). However, these alkylating compounds, particularly lomustine, proved highly myelosuppressive, often to the point that treatment cycles had to be halted and postponed.[11] However, studies evaluating the toxicity of TMZ demonstrated increased tolerance and improved quality of life compared with procarbazine-lomustine-vincristine therapy.[44–46]

Side effects of concomitant and adjuvant TMZ include nausea (which can be controlled by antiemetics)[47] and fatigue. Like its nitrosourea precursors, TMZ may also induce hematologic toxicity, although usually reversible and occurring in only 7% of patients.[14] Neurotoxicity may also manifest in response to TMZ, with acute events being associated with late neurotoxicity and a poorer OS.[48] However, grade III and IV adverse reactions typically present as thrombocytopenia, neutropenia,[44,49] and lymphopenia,[47] with reports of patients being at elevated risk for opportunistic infections, particularly *Pneumocystis carinii*.[14,50] In a review of TMZ-related infections, one study reported on the most frequently occurring diseases, including mucocutaneous candidiasis (28%); herpes zoster (13%); herpes simplex virus (10%); cytomegalovirus (13%); *P carinii* pneumonia (8%); hepatitis B virus (5%); and others (23%).[51] Another study suggested that higher doses of TMZ given in seven 14-day cycles led to lymphopenia in slightly more than half of their patients; however, it did not result in any infectious complications.[52] Similarly, no *P carinii* infections were reported in a children's study involving lymphopenia as a side effect of TMZ.[53] Overall, TMZ is very well-tolerated, with only 13% of patients discontinuing concomitant doses and 8% interrupting adjuvant doses because of toxicity.[14]

According to studies based on self-reported questionnaires, patients undergoing chemotherapy reported equivalent or greater quality of life (physical, emotional, social, and cognitive abilities) when under treatment with TMZ. These results were also reflective of improvement in health status because of the effectiveness of TMZ. Interestingly, quality of life status changed several weeks before disease progression, which was later measured by MRI and CT scans.[45,54]

However, a unique side effect of TMZ is pseudoprogression, the transient enhancement of diseased areas on MRI that gives the impression of tumor progression despite the overall benefit of radiotherapy and chemotherapy.[55–57] Pseudoprogression often precedes recovery and is believed to be inflammation or necrosis caused by radiation therapy and chemotherapy. TMZ cycles should be continued even if pseudoprogression occurs,[57–60] and only after three cycles should treatment be reevaluated.[61–63] Several

studies report that up to 50% of progression cases are pseudoprogression,[62,63] which can be alleviated with continued TMZ administration (**Fig. 3**). As with the assessment of quality of life, the complication of pseudoprogression necessitates a more effective method of evaluating disease progression.

DOSAGE AND SCHEDULING

Because TMZ degrades within hours[29] and its effects are dose-dependent, patient adherence to dosing schedules is integral to treatment. In newly diagnosed glioblastoma, TMZ is administered concomitantly with radiation therapy at 75 mg/m^2 per day for 42 days. It is then given as an adjuvant in cycles for 5 days, each 28-day period. Dosing for the first adjuvant cycle is 150 mg/m^2 per day for 5 days, with subsequent cycles two to six at 200 mg/m^2 per day. For recurrent glioblastoma with no prior chemotherapy, dosing is 200 mg/m^2 per day for 5 days, each 28-day period. In recurrent glioblastoma with prior chemotherapy, dosing is 150 mg/m^2 per day for 5 days, each 28-day period.[64] However, alternative schedules exist and should be modified if hematologic toxicity becomes severe.

Activation of tumor cell death is an obvious goal of chemotherapy. However, several studies have suggested that TMZ may confer a cytostatic effect on a significant percentage of GBM cells, rather than a cytotoxic one. Investigations of saturating cells in 500 to 1290 μM of TMZ found 30% to 40% cell survival.[38,65,66]

One study reexamined the extensive length of dosing during concomitant treatment, claiming

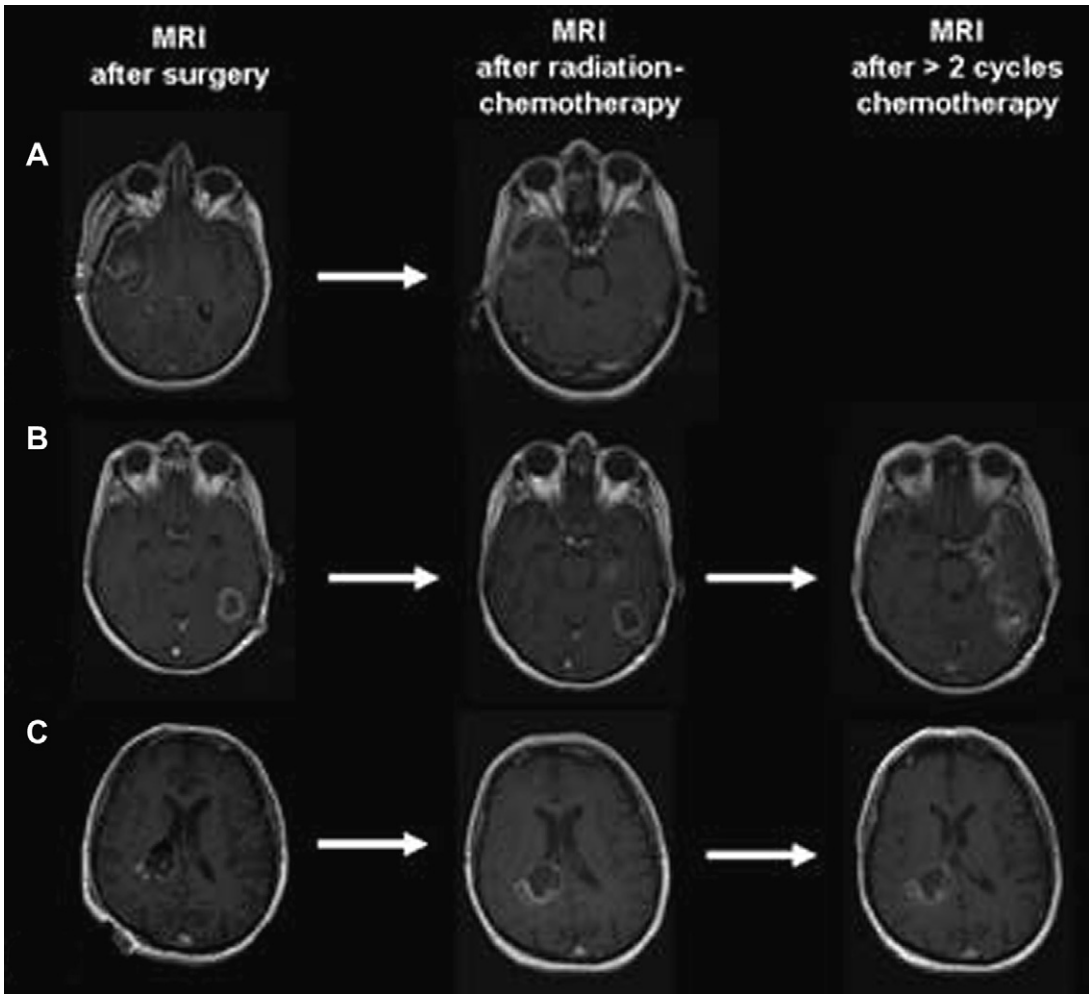

Fig. 3. T1-weighted contrast-enhanced MRI of glioblastoma demonstrating (*A*) partial response, (*B*) disease progression, and (*C*) pseudoprogression. (*From* Roldan GB, Scott JN, McIntyre JB, et al. Population-based study of pseudoprogression after chemoradiotherapy in GBM. Can J Neurol Sci 2009;36:617–22; with permission.)

that TMZ is effective even if only given during the first and last weeks of radiation therapy.[67] According to their results, median OS was reportedly 18 months, and 2-year survival rate was 34.8%. However, only 25 glioblastoma patients were included in the study, and no statistics were provided on the significance of their results compared with traditional dose scheduling. Another more extensive study compared two dose regimens of TMZ. Group A was given 50 mg/m^2 for 5 days each week on radiotherapy, whereas group B was given 75 mg/m^2 for 7 days each week on radiotherapy. Subsequently, 2-year survival rate for group A was 43%, whereas group B's 2-year survival rate was 49%, with no statistically significant difference found between groups.[68]

A study on adjuvant TMZ therapy showed that alternative dose-dense schedules (150 mg/m^2 daily for Days 1–7 and 15–28 of each 28-day cycle) confer benefits greater than standard dosing schedules, whereas metronomic adjuvant dosing (50 mg/m^2 daily, every day of 28-day cycle) did not provide any additional advantage. Two-year survival was 34.8% for dose-dense adjuvant treatment and 28% for metronomic schedules. Median OS for the dose-dense and metronomic treatments was 17.1 months and 15.1, respectively.[69] These findings reiterate the Norton-Simon model of cellular proliferation[69,70] and suggest that a dose-dense regimen may prevent glioma cells from proliferating between cycles. Although the effectiveness of alternative dosing schedules continues to be debated and requires further investigation, target dosage should minimize hematologic toxicity and avoid development of drug-resistance.[69]

Patient adherence to dosing schedules is critical in that they are designed to sufficiently overwhelm and deplete the glioma's O^6-methylguanine-DNA methyltransferase (MGMT),[14] a protein that repairs DNA by removing methyl groups from the O^6 position of guanine residues[71] and becomes permanently inactivated while reversing TMZ-induced DNA methylation. In patients with hypermethylated MGMT promoter regions, subsequent decreased protein expression leads to a reduction in DNA repair and increased tumor cell destruction.[12] General consensus throughout the literature suggests that MGMT protein expression confers an increased propensity for TMZ resistance up to 10-fold.[38,65,72]

Epigenetic modulation of the MGMT promoter gene by hypermethylation results in decreased MGMT mRNA expression and increased response to TMZ therapy (**Fig. 4**).[73–76] Hegi and colleagues[71] demonstrated a 21.7-month median OS in MGMT methylated patients treated with adjuvant TMZ compared with 12.7 months in patients with unmethylated promoter regions. Similarly, in MGMT-negative cells, radiotherapy with concomitant TMZ led to significant increase in survival compared with radiotherapy with adjuvant TMZ. MGMT-positive cells, however, showed no difference in survival regardless of treatment arm, supporting the significance of MGMT status in TMZ effectiveness.[19] Results remain pending from a phase III trial in which more than 1100 patients with newly diagnosed GBM received either standard-dose TMZ or dose-intensive TMZ in addition to standard therapy. This study aims to evaluate the potential of TMZ to deplete MGMT activity and improve overall patient survival.[12]

In another study involving 63 high-grade gliomas, MGMT mRNA expression was found to be a strong prognostic marker, because low levels

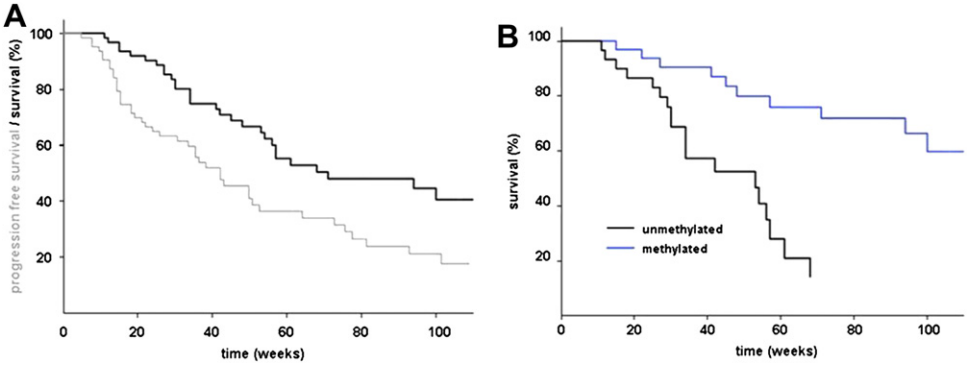

Fig. 4. Kaplan-Meier diagrams depicting (*A*) progression-free survival and overall survival of 63 patients with malignant gliomas, and (*B*) overall survival of patients according to MGMT promoter methylation status. (*From* Kreth S, Thon N, Eigenbrod S, et al. O6-Methylguanine-DNA methyltransferase (MGMT) mRNA expression predicts outcome in malignant glioma independent of MGMT promoter methylation. PlosOne 2011;6(2):e17156; with permission.)

was associated with prolonged time to progression, increased treatment response, and improved survival. Furthermore, mRNA expression was also correlated with MGMT promoter methylation status in most patients, and in cases of discordant findings between epigenetic and expression profiles (eg, hypermethylation with increased MGMT mRNA), MGMT mRNA was still strongly prognostic. Given evidence of incongruent findings related to methylation status and MGMT expression, methylation-independent pathways may necessitate further evaluation to elucidate the mechanisms behind MGMT mRNA expression.[75]

Sensitivity and effectiveness of TMZ treatment may be predicted by screening for epigenetic methylation of the promoter gene for MGMT. Higher methylation translates to silencing of MGMT, which is prognostic for benefit from TMZ treatment.[60,71,77–80] These patients have demonstrated a 2-year survival rate of 46%,[63] whereas only 13.8% of patients who lack such methylation survive beyond 2 years.[63,71,81–86]

TMZ AND RADIATION THERAPY

Several studies have suggested the synergistic properties of TMZ and radiotherapy.[19,65,71,87] It has been established that glioma cells under the assault of TMZ are arrested in G_2/M phase of the cell cycle, hindering them from subsequent growth and division,[40,88,89] and rendering them sensitive to radiotherapy.[89] Tsien and colleagues[90] reported findings in their study using intensity modulated radiotherapy of 75 Gy in 30 fractions, resulting in a median OS of 20.1 months. One recent evaluation of data was collected from nearly 14,000 patients throughout the United States comparing outcomes before and during the TMZ era. Before TMZ (2000–2003), patients treated with surgery and radiation therapy survived a median 12 months, whereas those within the 2005 to 2008 TMZ treatment years had a median survival of 14.2 months. However, outcomes were found to dramatically vary by age, reporting a 31.9-month survival for patients in the 20 to 29 age range, whereas those older than 80 years displayed a 5.6-month survival. Similar findings were reported by Darefsky and colleagues[91] regarding a database of nearly 20,000 patients. These findings confirm the treatment effect of TMZ in a population-based cohort.

Although the postoperative standard of care has traditionally contained fractionated irradiation doses of 60 Gy, one study evaluated the use of accelerated radiotherapy with 1.8 Gy twice daily to a total dose of 54 Gy within 3 weeks. They demonstrated that the addition of TMZ was found to prolong median OS from 11.3 to 16 months compared with those without chemotherapy. This OS was found to be similar to that of TMZ added to conventionally fractionated radiotherapy, making it a reasonable alternative. This approach has been suggested for patients with a severely limited life expectancy. However, because outpatient treatment may be quite burdensome given the frequency of treatments, considerations for quality of life should be a point of discussion.[92]

TMZ AND IMMUNOTHERAPY

Regulatory T cells may modulate the native immune system through various mechanisms. They can secrete immunosuppressive cytokines (transforming growth factor-β and interleukin-10), or may be constitutively activated and inhibit cytotoxic T lymphocytes.[93,94] In patients with melanoma, the Treg population has been shown to become decreased after prolonged exposure to low-dose TMZ.[93,95] This modulation of the GBM microenvironment may have profound effects on immunoreactivity against tumor cells. Given that radiation therapy and TMZ therapy may alter the regulatory and effector peripheral blood mononuclear cells, immunotherapeutic studies may need to consider the potential interactions of this altered immune system.[93] Furthermore, patients with GBM treated with dendritic cell vaccinations plus chemotherapy have demonstrated prolonged survival compared with those receiving the vaccination alone, with evidence suggesting that dendritic cell vaccinations may be capable of sensitizing tumor cells to the effects of TMZ.[93,96–98]

SUPPLEMENTAL, COMBINATION, AND ALTERNATIVE THERAPIES

Cancer stem cells are thought to constitute a subpopulation of tumor cells and mediate chemoresistant properties. Although the remaining tumor may undergo cell death after standard therapies, growing evidence suggests that these stem cells are responsible for GBM recurrence.[33,38] Resistance to TMZ may not only be based in genetic predisposition, but it can also be acquired, as in the case of MSH6 mutations that are found in posttreatment glioblastoma but not in pretreatment glioblastoma.[99] Given the substantial population of patients with resistance to TMZ treatment, the development of supplemental, combination, or alternative therapies is critical to optimize GBM management.

When MGMT is absent or becomes depleted, O^6-methylguanine may initiate mismatch repair (MMR) enzymes or downstream protein pathways, ultimately resulting in apoptosis and cell death. However, nearly all patients with GBM endure tumor recurrence, with most of these lesions being TMZ-resistant as a result of increased MGMT expression or decreased MMR.[100]

Although the TMZ-induced O^6-methylguanine lesion is thought to be associated with most of this chemotherapeutic's cell toxicity, the effects of N^3-methyladenine and N^7-methylguanine lesions have also been recently investigated.[100] Although N^3-methyladenine and N^7-methylguanine are thought to contribute a minimal effect on tumor destruction because of their rapid restoration by base excision repair (BER), BER inhibition may provide a potential target for applied therapeutics. The rate-limiting step in BER is thought to be DNA polymerase-β, and its inhibition has demonstrated accumulation of this pathway's substrates with increased cell sensitivity. Additionally, BER inhibition using methoxyamine hydrochloride has also been shown to increase the cytotoxic effects of N^3-methyladenine and N^7-methylguanine.[100–102]

It has been suggested that cell death after BER activation may be an energy-dependent phenomenon, because NAD^+ consumption for poly(ADP-ribose) synthesis after poly(ADP-ribose) polymerase (PARP) hyperactivation leads to ATP depletion. Alkylation sensitivity caused by failed BER may also be reversed by NAD^+ precursor supplementation, further supporting this proposed mechanism of action.[100,101] In a study by Goellner and colleagues,[100] investigators reported findings on a combined approach with inhibitors of BER (methoxyamine hydrochloride) and NAD^+ (FK866) pathways to enhance N^3-methyladenine and N^7-methylguanine tumor cell sensitivity to TMZ. They determined that TMZ resistance caused by either MMR deficiency or MGMT overexpression may be reversed by combined BER and NAD^+ biosynthetic inhibition. However, particular concern may arise from depleting NAD^+ and ATP from healthy tissue, resulting in necrotic cell death of normal parenchyma. Yet, it has been suggested that given the high energy demands of rapid tumor cell growth, the combination therapy may selectively affect GBM cells.[100,103]

In addition, PARP inhibitors may also prove beneficial in the restoration of TMZ sensitivity in MGMT unmethylated GBM, because PARP enzymes are involved in base-excision repair after TMZ N^3 and N^7 methylation. Such inhibitors have demonstrated in vitro and in vivo abilities to induce TMZ sensitivity in previously resistant glioma tumor cells.[12,104]

Another DNA repair enzyme,[45] O^6-alkylguanine-DNA alkyltransferase, plays a similar TMZ-resistant role, yet no longer remains an obstacle to treatment because its effects have been shown to be effectively inhibited by O^6-benzylguanine.[19,21,40,105,106]

Recent studies indicate that supplements, such as interferon-β, may sensitize glioma cells to TMZ treatment and can extend median OS to 19.9 months compared with TMZ-only group of 12.7 months.[107] One-year survival rates were 83.6% for the interferon-β/TMZ combination group and 67.6% for standard TMZ treatment, whereas 2-year survival rates were 34.5% and 22.1%, respectively. These effects were thought to result from the antiproliferative cascades involved with interferon-β.[107] Furthermore, other agents, such as sphingosine kinase inhibitors,[108] have also been shown to modulate and resensitize resistant gliomas to TMZ.

Current TMZ clinical trials are in pursuit of combination therapies that help to improve its effectiveness. Recent developments that target unregulated glioma growth include inhibition of tumor growth factors,[109] angiogenic agents,[110] VEGF signaling, epidermal growth factor receptors,[111] PKC/phosphatidylinositol 3-kinase (PI3K)/AKT pathways, SRC-family kinases, platelet-derived growth factor receptor, integrins, c-MET, glutamate receptors, and histone deacetylase.[1]

PI3K has been associated with cell survival, growth, and proliferation, and its regulators have been found to be mutated at a high frequency in GBM tumors. Evaluation of 209 clinical GBM samples found that 86% displayed activating mutations or genetic amplifications in the RTK/PI3K pathway.[112,113] GBM also commonly displays a mutation or loss of PTEN, a tumor suppressor that modulates the activity of PI3K and is found to be mutated or deleted in 36% of clinical GBM samples.[112,113] These PTEN alterations result in constitutively activated PI3Kinase, subsequent activation of mTOR, and resultant tumor growth and resistance to radiation.[12] Preclinical studies have suggested that inhibitors of PI3K or its downstream signaling proteins (including AKT, GSK-3, and mTOR) may confer G1 arrest of GBM cell lines in vitro and increased TMZ sensitivity in vivo.[1,114–116] In one preclinical study, a combination inhibitor of PI3K/mTOR (XL765) was used to determine the effects on intracranial, orthotopic glioma xenografts as a single agent, and as a synergistic addition to TMZ. The dual inhibition of the PI3K/mTOR pathways may be particularly important in GBM tumors, because mTOR inhibition may induce a negative feedback loop that results in increased PI3K/Akt activity. Authors of this study found that XL765 resulted

in concentration-dependent cell death; inhibition of the PI3K/mTOR pathways; a decreased median tumor bioluminescence by 12-fold (indicating decreased tumor burden); and increased survival. Although TMZ alone resulted in a 30-fold decreased bioluminescence, the combination of TMZ and XL765 produced a 140-fold reduction and a trend toward improved survival compared with TMZ alone.[112] These findings indicate the potential for future phase Ib/II trials to evaluate the clinical benefit these pathway modulators may possess.

Integrins have been associated with metastasis and angiogenesis, because they play important roles in cellular adhesion, migration, and invasion. The α_v integrin inhibitor, cilengitide, has been evaluated in clinical trials reporting promising 6-month PFS rates (69%), 12-month OS (68%), 24-month OS (35%), and median OS (16 months), with particularly increased PFS and OS rates for patients with MGMT promoter methylation.[117]

Within the pediatric population, it has been discovered that malignant gliomas may be genetically distinct from those of adults, displaying a markedly decreased frequency of epidermal growth factor receptors amplification, PTEN alterations, and IDH gene mutations.[118–120] In a study evaluating 107 pediatric patients with high-grade gliomas, TMZ failed to provide any added survival benefit compared with the use of other chemotherapies during standard treatment regimens. This suggests that GBM present in pediatric patients may represent a unique entity from that of adults, necessitating age-appropriate therapeutic options.[118]

FDA-APPROVED THERAPIES

Other FDA-approved therapies for glioblastoma include BCNU wafers (approved in 1996); bevacizumab (approved in 2009); and NovoTTF-100A (approved in 2011). BCNU wafers are

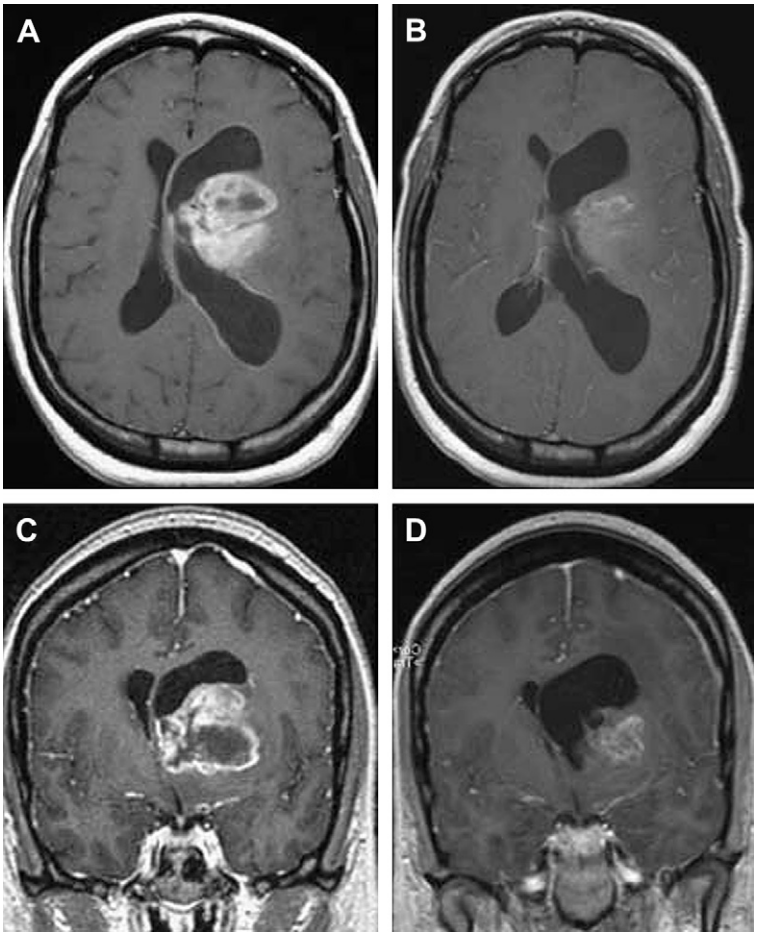

Fig. 5. Postcontrast axial and coronal T1-weighted MRIs at baseline (*A*, *C*) and after treatment with bevacizumab and irinotecan (*B*, *D*). (*From* Vredenburgh JJ, Desjardins A, Herndon JE II, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 2007;25(30):4722–9; with permission.)

a combination of carmustine chemotherapy and a polymer wafer that are administered directly into the tumor resection cavity. This therapy was approved by the FDA in 1996 for the treatment of recurrent GBM after a phase III double-blind study involving 222 patients demonstrated more than a 50% increase in 6-month survival.[121,122] After a meta-analysis of two phase III trials depicting an improved median survival of 13.1 months versus 10.9 months in the control group, FDA approval was granted in 2003 for newly diagnosed high-grade gliomas.[122–124] Thus, BCNU wafers may provide a modest treatment during the interval between surgical resection and other adjuvant therapies.[12]

Bevacizumab is a monoclonal antibody for VEGF-A that inhibits proliferation of endothelial cells and angiogenesis.[125] This therapeutic approach has been widely prescribed as treatment for other tumor types, including colorectal, breast, and lung cancers. However, its precise role for glioblastoma remains elusive. Although many studies have demonstrated improvement in radiologic response and 6-month PSF, others have failed to identify any increase in OS. However, recent findings have suggested significant benefit to patient outcomes with the administration of bevacizumab, implying the necessity for additional studies to evaluate this treatment modality (**Fig. 5**).[125–146]

NovoTTF-100A is a noninvasive external electrode system that generates "tumor treatment fields" (TTF) or alternating electric fields that arrest cell proliferation and induce apoptosis. They inhibit the proper formation of mitotic spindles, cause cells to burst when dividing,[147] and interfere with organelle assembly.[148] A phase III clinical trial showed that treatment with TTF (120 patients) had better responses and tolerance than treatment with the "best standard chemotherapy" with TMZ (117 patients). Median OS for TTF treatment was 6.6 months, compared with median OS of 6 months with chemotherapy.[148] However, the results were not statistically significant, indicating that whereas NovoTTF-100A was not a better alternative to chemotherapy, it could be considered comparable.

NovoTTF-100A offers advantages over TMZ, because the system can be used at home and lacks the side effects commonly associated with chemotherapy. Because the electric fields cannot penetrate bone, this treatment can preserve the integrity of bone marrow and other blood cells that may become injured as a result of hematologic toxicity in TMZ therapy.[147,149] NovoTTF-100A side effects include dermatitis at electrodes sites in 17% of patients[147,148,150–152] and the theoretic risk of cardiac arrhythmia or seizures; however, these have yet to be documented in clinical trials.[6,147] NovoTTF-100A holds promise as an alternative therapy for patients who are poor surgical candidates or are unable to tolerate TMZ chemotherapy.

SUMMARY

Several advancements have been made in the treatment of glioblastoma. Benefits of TMZ include increased tolerance and improvement in OS compared with surgery alone, radiation therapy alone, or first-generation alkylating agents. Despite these advancements, myelosuppression, acquired tolerance, and dismal 2-year survival rates remain as reminders of current therapeutic limitations. Recent years have revealed novel innovations, including such therapies as BCNU wafers, bevacizumab, NovoTTF-100A, and a variety of potential adjuvants to TMZ treatment. Ultimately, it may be necessary to combine several of these approaches to best optimize the management of glioblastoma. A recent study reported that TMZ may be combined with as many as three other agents to target growth of malignant cells.[20,52] However, the potential toxicity of these multimodality treatments must be kept in mind to minimize treatment-related complications.[153] Although TMZ has provided an improvement in the management of GBM, its conferred advantage is by no means sufficient alone. Future research is necessary to augments its effects and optimize patient outcomes.

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