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TOPIC REVIEW

Temozolomide treatment outcomes and immunotherapy efficacy in brain tumor

Kelly M. Hotchkiss[1] · **John H. Sampson**[1]

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Abstract

Introduction Glioblastoma (GBM) has a survival rate of around 2 years with aggressive current standard of care. While other tumors have responded favorably to trials combining immunotherapy and chemotherapy, GBM remains uniformly deadly with minimal increases in overall survival. GBM differ from others due to being isolated behind the blood brain barrier, increased heterogeneity and mutational burden, and immunosuppression from the brain environment and tumor itself.

Methods We have reviewed clinical and preclinical studies investigating how different doses (dose intense (DI) and metronomic) and timing of immunotherapy following TMZ treatment can eradicate tumor cells, alter tumor mutational burden, and change immune cells.

Results Recent clinical trials with standard of care (SoC), DI and metronomic TMZ regimes are no able to completely eradicate GBM. Elevated TMZ levels in DI treatment can overcome MGMT resistance but may result in hypermutation of surviving tumor cells. Higher levels of TMZ will also generate a higher degree of lymphopenia compared to SoC and metronomic regimes in preclinical studies.

Conclusion The different levels of lymphopenia and tumor eradication discussed in this review suggest possible beneficial pairings between immunotherapy and TMZ treatment. Treatments resulting in profound lymphopenia will allow for expansion of vaccine specific T cells or of CAT T cells. Clinical and preclinical studies are currently comparing different combinations of TMZ and immunotherapy timing to treat GBM through a balance between tumor killing and immune cell expansion. More frequent immune monitoring time points in ongoing clinical trials are crucial for further development of these combinations.

Keywords Chemotherapy · Temozolomide · Glioblastoma · MGMT · MMR · Immunotherapy

Introduction

Cancer is a devastating disease characterized by rapid uncontrolled division of mutated malignant cells. Primary brain cancers are particularly tragic with low overall survival rates. Gliomas, including ependymomas, oligodendrogliomas, and astrocytomas, are the most common malignant brain tumors in adults. Glioblastoma (GBM, glioma WHO grade IV) is the highest-grade astrocytoma and affects 3.19 in 100,000 adults in the United States [1]. The highly infiltrative nature of GBM results in difficulty removing the entire malignancy therefore surgery alone not a viable treatment process and allows for recurrence in patients not receiving additional treatment. Despite intense treatment strategies including surgery, radio- and chemotherapy as well as tumor treating fields (TTF) to date patients with GBM have a median survival of less than 24 months and a 5-year survival rate of just 2–4% [2, 3]. These short survival expectations compared to other cancers has drawn significant interest from scientist developing novel and combinatorial therapies to improve patient outcomes. Immunotherapy strategies including checkpoint blockade, DC and peptide vaccine, and CAR-T cells are currently in clinical trials for GBM and other tumors [4–8]. Impressive results with monotherapy and combination treatment with chemotherapy, radiation or immunotherapy have been found in liquid tumors and some solid tumors outside of the CNS. However, recent clinical trials implementing these strategies for GBM have resulted in no survival benefit [9–11].

The failure of current treatments to eradicate malignant brain tumors similarly to other solid tumors can be attributed

✉ John H. Sampson
john.sampson@duke.edu

[1] Duke University, Durham, NC, USA

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to both the natural physiology of the central nervous system (CNS) and characteristics of GBM itself. Events occurring within the CNS are pseudo-isolated behind the blood brain barrier (BBB), an evolutionary mechanism that protect against toxins, foreign pathogens, and systemic inflammation [12]. The BBB is a semipermeable membrane, which limits passage of solutes, immune cellular migration, and humoral diffusion in an effort to protect the brain. This membrane can limit passage of specific pharmacological treatments to interact directly with tumors. While passage into the brain is tightly regulated immune surveillance does occur and is increased during times of inflammation and through recently discovered classical lymphatic system within the CNS allowing for the immune system to enter and leave the brain [13–15].

Another hurdle for current anti-tumor strategies is the significantly less immunogenic nature of GBM compared to other tumors such as melanoma. The lack of immunogenicity is in large part due to reduced target antigens on the surface, which lead to inadequate T cell activation and present a challenge for immunotherapy development. Reduced tumor specific and overall heterogeneity of GBM make it difficult to select vaccine antigens to target a majority of tumor cells. Tumors also produce a variety of immunosuppressive molecules, which then become isolated within the microenvironment and concentrated in the CNS. These factors actively convert immune cells to a tumorigenic phenotype [16]. These molecules include transforming growth factor beta (TGF-β), interleukin 10 (IL-10), and indoleamine 2,3-dioxygenase (IDO), all which reduce an immune response [16–18]. These factors generate immunosuppressive cell phenotypes including: regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAMs); which collectively contribute to tumor angiogenesis and limit innate and adaptive antitumor immunity [19].

Temozolomide (TMZ) is the most commonly used chemotherapeutic agent used in GBM. However some patients show resistance link with isocitrate dehydrogenase 1 (IDH1) mutations, as well as high levels of methylation of the MGMT (O6-methyguanine DNA methyltransferase) promoter [2, 20, 21] which lead to reduced efficacy in standard TMZ treatments. TMZ treatment has been shown to directly target tumor cells and have reasonable toxicity at increased doses. Studies designed to increased chemotherapy load in a patient have used higher doses for short time frames as well as lower sustained doses. Different doses of TMZ can alter DNA repair mechanisms and overall tumor genetic profile while also changing composition of immune cells surrounding the tumor. In this review, we will discuss how three outcomes of TMZ treatment (Fig. 1) can influence immunotherapy efficacy to overcome or succumb to current limitations in GBM treatment.

TMZ directly kills tumor cells to reduce tumor burden

The current standard of care for initially diagnosed GBM consists of maximal safe surgical resection followed by adjuvant chemoradiation. Chemotherapeutic drugs are commonly used to treat a variety of tumors due to their ability to target and induce death in rapidly proliferating cells, like in cancer. These drugs can be divided into several categories based on their mechanism of action (1) alkylating drugs; (2) antimetabolites; (3) topoisomerase inhibitors; (4) microtubular poisons; and (5) cytotoxic antibiotics. The most common and successful chemotherapeutic in GBM is the lipophilic, monofunctional prodrug temozolomide (TMZ) which belongs to the alkylating group known to arrest cell cycle at G2/M and eventually lead to apoptosis [22]. TMZ is absorbed intact at acidic pH and rapidly breaks down to form monomethyl triazene 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MITC) at physiological pH > 7. This molecule further reacts in water to form 5-amino-imidazole-4-carboxamide (AIC) and the methyl diazonium cation [23]. This cation mediated TMZ toxicity by preferentially adding methyl groups to DNA at N^7 and O^6 positions on guanines and N^3 regions on adenine. Unrepaired O^6 methylation of guanine (O^6-meG) adducts are cytotoxic [24].

In standard treatment, resection is followed by fractionated focal radiotherapy administered at 2 Gy per fraction once daily 5 days per week over a 6-week period, resulting in an accumulated dose of 60 Gy. Concomitant with radiotherapy, temozolomide (TMZ) chemotherapy is given daily at a dose of 75 mg/m^2 over the 6-week period. After a 4-week break, patients then receive maintenance TMZ on days 1–5 every 28 days for 6 cycles at a dose of 150–200 mg/m^2 [2]. While all patients now receive the standard TMZ and radiotherapy, clinical trials have begun to modify the adjuvant chemotherapy schemes in hopes of extending PFS and OS in GBM. TMZ dose can be increased by elevating the dose or extending the number of consecutive days treatment is given or both. Dosing schedules where patients receive TMZ for "three weeks on-one week off", "one week on-one week off", "continuous dosing for 6-weeks" and others have doubled TMZ levels while maintaining acceptable toxicity [25–27].

The efficacy of increased dose in one week on–one week off strategy following standard RT-TMZ was assessed by Wick et al. in a single-arm, non-randomized trial with 90 adult patients with recurrent gliomas, 64 of which were glioblastoma. Patients received 150 mg/m^2/day on days 1–7 and 15–21 for 4 weeks. Results were compared to historical studies where patients received the standard 150 mg/m^2 for 5 days. 11 patients developed grade 4 lymphopenia but overall, the treatment did not result in cumulative lymphopenia or

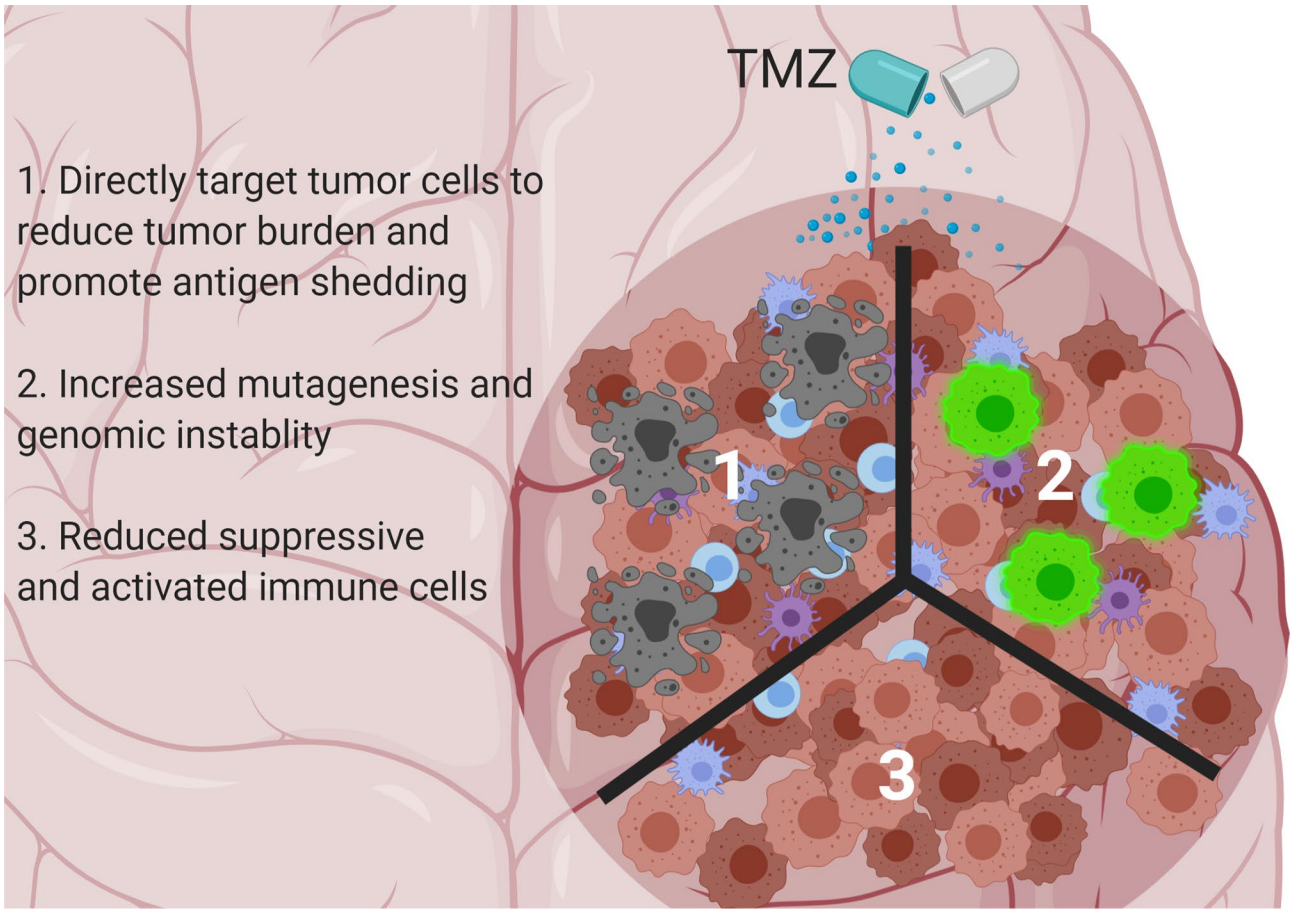

Fig. 1 Temozolomide can have both beneficial and detrimental effects on immunotherapy efficacy by (1) reducing tumor burden by directly killing GBM, (2) increasing mutagenesis in surviving tumor cells and (3) resetting immune response by eliminating both suppressive and activated immune cells throughout the body

opportunistic infections. Patients in this study had a median PFS of 24 weeks, with 43.8% PFS at 6 months [28]. Median OS was 38 weeks and 12-month OS was 23%. Data from this trial suggest that the alternating weekly schedule of temozolomide showed clinically meaningful improvement in survival outcomes compared to historical trial (PFS rate at 6 months: 21%). This study showed that the alternating-weekly schedule is feasible, safe, and effective and recommended further investigation of this strategy in randomized studies [28].

In an effort to generate less toxic and more tolerable treatment clinicians and researchers have used low doses for extended period of time following standard RT-TMZ, also known as metronomic dosing. This dosing strategy can be beneficial for patients who are not able to handle the increased or even standard level of TMZ without adverse effects [29]. The main goal of this dosing strategy was to optimize antitumor efficacy by targeting tumor vasculature rather than tumor cells directly [30, 31]. In 2006 Kong et al. showed a metronomic TMZ dose strategy may provide increased survival in patients refractory to standard TMZ. In this pilot study, patients received 40 mg/m^2 TMZ daily. The overall and progression free survival were 11.0 months and 6.0 months from treatment start date, respectively [32]. Additional studies have shown metronomic dosing strategies to extend PFS even in patients who had previously undergone extensive treatments.

While chemotherapy at any current dose strategy is not able to eradicate GBM alone, it may provide a helpful starting point to boost immunotherapy efficacy. Less tumor cells alive means fewer cells immunotherapy needs to target and slowed tumor growth. Additionally, as tumor cells die they will release antigens which can be used by the host immune system to generate immune responses to combat the disease [33]. This allows for an excellent opportunity for immune based treatment intervention.

TMZ can cause increased mutations in normal non-immunogenic GBM

TMZ is given to patients as standard of care; however, 55% of GBM patients are resistant to treatment due to their MGMT DNA repair system. Two major mechanisms counteract the cytotoxic activity of TMZ methylation of guanine (O^6-meG), MGMT and mismatch repair (MMR) deficiency. MGMT counteracts the apoptotic effects of alkylating reagents by transferring a methyl group from guanine to repair DNA damage and prevent cell death. In the absence of MGMT expression, the MMR pathway is triggered by the mismatches in base pairs following TMZ methylation. Within this pathway, MSH2, MSH6. MLH1, and PMS2 proteins recognize and bind to mismatched guanine, forcing cells to enter a cycle of DNA repair [34]. The MMR pathway allows for newly synthesized daughter DNA strands to be repaired while parental stands remain methylated. The difference between parental and daughter DNA strands leads to a cycle of futile repair and subsequent DNA breaks, cell arrest, and death. Cells without proficient MMR pathways gain tolerance to DNA base pair mismatch and acquire genetic hypermutation [35].

Dose-intensified (DI), or dose-dense (DD), levels of TMZ have been of interest to improve overall survival by potentially overcoming MGMT resistance. Unfortunately, even patients who initially respond to TMZ treatment can become resistant overtime allowing for incomplete tumor killing and recurrence. TMZ treatment will disrupt DNA repair pathways and can increase mutagenesis and genomic instability in surviving tumor cells [36]. Several longitudinal observational studies have compared gliomas before and after the patient underwent standard of care treatment. These studies have established at least two distinct genomic outcomes to exist upon recurrence; hypermutant and non-hypermutant [37–39]. A non-hypermutant recurrent GBM will have a similar tumor mutational burden and genomic diversity as the primary tumor. Hypermutant recurrent GBM are characterized by increases in subclonal mutation, enrichment of signature TMZ mutagenesis with higher levels of C:G over T:A mutations, and inactivating mutations in the MMR pathway molecules [37]. A majority of GBM will recur as non-hypermutant with only around 10% recurring with a higher mutational load. Hypermutant recurrence was initially observed patients with low-grade astrocytomas following initial TMZ treatment [40], however it has now been seen to a lesser extent in patients with grade IV GBM [39, 41]. The higher potential for low-grade glioma to recur as hypermutant tumors compared to high-grade gliomas raises interesting questions about what mechanisms are involved in hypermutation recurrence. This different potential for mutagenesis is present even with stable levels of MGMT between both low- and high-grade gliomas which suggests the importance of MMR in addition to levels of MGMT [42].

A recent study comparing the expression of MMR proteins between matched primary and recurrent GBM treated with standard RT-TMZ showed a downregulation of MMR genes in recurrent tumors [43]. The specific cytotoxic activity of TMZ relies on active proliferation of cells and rapid DNA replication. As mentioned before, proliferating cells lacking appropriate MMR repair will ultimately generate increased mutations in response to TMZ treatment. This suggests that slower proliferation could be protective against developing a hypermutant recurrence phenotype; however, this may also reduce the effectiveness of TMZ specific killing of tumor cells. By increasing the number of mutations and potential for genetic diversity within a tumor, TMZ treatment can limit the effectiveness of antigen targeting immunotherapies such as DC vaccines and CAR T cells. Therefore, it is important for studies to assess the effects of time of TMZ dose following radiation treatment to establish a beneficial balance between proliferation and dormancy of tumor cells. An additional method for preventing the development of hypermutant recurrence could be to screen patients for levels of MMR prior to repeated TMZ treatment. In a recent retrospective study published in Nature, Struve et al. suggest a link between EGFRvIII expression and level of MMR proteins in tumor cells with less MMR expression in the absence of EGFRvIII. This findings may be beneficial in screening patients who will respond favorably to TMZ treatment [44] without generating increased hypermutations.

TMZ changes immune cell profile

While clinical and preclinical studies manipulating chemotherapy dose alone have thus far been unsuccessful, knowledge on how chemotherapy alter the immune system can be used to improve efficacy of different immunotherapeutic treatments. Proliferating immune cells, such as activated T cells, can undergo apoptosis in a similar fashion to cancer cells. Studies have shown a rapid expansion of immune cells immediately following ablation due to TMZ treatment. Changes in TMZ doses and timing of immunotherapy administration following TMZ can be used to take advantage of the immune cell expansion phase. Several clinical [45] and preclinical [46] studies have highlighted the potential to exploit TMZ induced lymphodepletion to generate stronger immune responses while suppressive cells are ablated. The reduction of the classical immunosuppressive immune cells within a tumor environment can allow for the expansion of T cells following antigen specific activation through APCs [47]. Researchers have observed the effect of lymphodepletion to reset the host immune system and eliminate tolerance towards autologous tumor antigens. Therefore vaccinations given immediately following lymphodepleting can

have enhanced and prolonged antitumor efficacy, and TMZ regimens which generate complete immune depletion may be most beneficial [48]. The ability of TMZ to ablate tumor cells and immunosuppressive monocytes while not affecting professional APCs, DCs, suggests a beneficial role of chemotherapy with cancer vaccines.

Standard TMZ treatment plus radiation in GBM patients results in gross lymphopenia with drastic reductions in CD4+ T cells and CD8+ T cells and B cells to a lesser extent [49–51]. In addition to lymphocyte depletion, selective monocyte depletion has also been measured with standard TMZ treatment [52]. Fortunately, monocyte derived DCs are not targeted due to an increase in MGMT levels during maturation. These side effects can be modulated based on selection of drug and dosing strategy, unfortunately many clinical trials have a primary outcome of progression free survival (PFS) and overall survival (OS) and do not carefully characterize the effects on immune cell populations. The lack of immune monitoring in clinical trials leaves most of the information about the role of TMZ dose on immune cell populations to be discovered in preclinical animal models.

A preclinical study in rats tested a calculated standard TMZ dosing (30 mg/kg for 5 days) or DI (10 mg/kg for 3 weeks) to different low dose metronomic regimes (2 and 0.5 mg/kg for 3 weeks) in a chemotherapy resistance glioma model [53]. The low metronomic dose regime of 0.5 mg/kg for 3 weeks resulted in a significant decrease in circulating Treg/CD4 T cell ratio in spleens while the standard and higher dose regime did not. Additionally, the 0.5 mg/kg dose strategy also reduce immunosuppressive function of the remaining Tregs upon in vitro stimulation. A recent study by Karachi et al. aimed to characterize chemotherapy induced lymphopenia following both standard and metronomic TMZ as well as the resulting impact on clinically relevant immune checkpoint antitumor efficacy in mice [54]. Standard TMZ (50 mg/kg for 5 days) reduced both CD4+ and CD8+ T cells compared to metronomic (25 mg/kg for 10 days). Additionally, the standard TMZ regime resulted in greater CD8+ T cell exhaustion and poorer overall outcomes in PD-1 antibody tumor studies, while lower TMZ doses maintained cytotoxic T cell activity and direct tumor killing. The selection of metronomic doses is not standardized between animal models and An equivalent low dose used in these studies has not been tested in humans, but these do support future trials with metronomic dosing strategies. In Sampson et al., our group made the significant finding that increased host and vaccine induced antigen specific immune responses were recorded following EGFRvIII targeted peptide vaccine in patients receiving higher (DI) TMZ treatment compared to standard levels. In this trial (NCT02772094) patients in the experimental arm received 180 mg/m^2 TMZ and had an overall survival of 22.9 months. This was interesting due to the profound lymphopenia and elevated Treg ratios produced by DI TMZ [55].

Lymphopenia generated by standard TMZ treatment can also benefit CAR T cell therapy by creating a niche for genetically engineered CAR T cell expansion and persistence. Increased antigen specific T cell proliferation due to homeostatic recovery following lymphopenia has been observed in preclinical studies with standard and DI TMZ treatments [46]. Preclinical studies in rats have found reduced PD-L1 expression on GBM with TMZ treatment [56]. These data suggest a reduced immunosuppressive profile following chemotherapeutic intervention.

Even potentially negative changes in immune profiles following TMZ treatment can be leveraged as targets to enhance immunotherapy efficacy. By selectively blocking Tregs in mice and humans with CD25 or IL2-receptor alpha chain monoclonal antibodies within TMZ-induced lymphopenia correlated with enhanced anti-tumor T cell responses. Reduced Treg levels were associated with an expansion of vaccine stimulated effector T cells following DC vaccine [57, 58]. Additionally, in animal models TMZ chemotherapy appears to increase cross-priming of tumor antigen-specific CD4 and CD8 T cells following vaccination with tumor antigen pulsed DCs [59].

By taking advantage of TMZ dose and timing of increased expansion of activated T cells researchers can generate strong antitumor immune responses to tumor antigens. However, if immunotherapy is given to soon following or during TMZ treatment the immune system will continue to be suppressed and not elicit a strong anti-tumor response. In order to determine optimal time frames for different dose and timing schemes of TMZ treatment greater immune monitoring for clinical trials is required to establish immune profiles.

Conclusion and future perspectives on TMZ immunotherapy combination treatments

TMZ is a valuable asset in the fight against GBM but researchers must find a balance between potential beneficial and hindering effects. While TMZ can help to reduce tumor load and promote antigen release and prestation; it may also create a more malignant tumor and suppress the host immune response. In order to create optimal immunotherapy and chemotherapy strategies trials should be gathering greater amounts of immune monitoring data to not rely as heavily on preclinical studies. Elements such as timing of both TMZ and initial immunotherapy intervention as well as dose of chemotherapeutic agent are crucial areas that should be further explored.

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Compliance with ethical standards

Conflict of interest The authors do not have any conflicts of interest.

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