Review

O\(^6\)-methylguanine DNA methyltransferase as a promising target for the treatment of temozolomide-resistant gliomas

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Temozolomide (TMZ) is an alkylating agent currently used as first-line therapy for gliomas treatment due to its DNA-damaging effect. However, drug resistance occurs, preventing multi-cycle use of this chemotherapeutic agent. One of the major mechanisms of cancer drug resistance is enhanced activity of a DNA repair enzyme, O\(^6\)-methylguanine-DNA-methyltransferase (MGMT), which counteracts chemotherapy-induced DNA alkylation and is a key component of chemoresistance. MGMT repairs TMZ-induced DNA lesions, O\(^6\)-meG, by transferring the alkyl group from guanine to a cysteine residue. This review provides an overview of recent advances in the field, with particular emphasis on the inhibitors of MGMT and underlying mechanisms.

Literature search was performed through PubMed and all relevant articles were reviewed, with particular attention to MGMT, its role in TMZ-resistant gliomas, effects of MGMT inhibitors and the underlying mechanisms. Several strategies are currently being pursued to improve the therapeutic efficacy of TMZ via inhibition of MGMT to reduce chemoresistance and improve overall survival. MGMT may be a promising target for the treatment of TMZ-resistant gliomas.

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Facts

- Temozolomide (TMZ) is an alkylating agent currently used as first-line therapy for gliomas treatment due to its DNA-damaging effect.
- Resistance to TMZ emerges with prolonged treatment, mainly due to O\(^6\)-methylguanine-DNA-methyltransferase (MGMT), which repairs O\(^6\)-methylguanine (O\(^6\)-meG) lesion by transferring the alkyl group from guanine to a cysteine residue, which posts a major therapeutic challenge.
- Inhibition of MGMT with several O\(^6\)-guanine derivatives and related compounds has been explored and shown to enhance TMZ-induced cytotoxicity in cancer cells.
- There is a challenge in overcoming dose-limiting myelosuppressive toxicity of TMZ while maintaining its efficacy.
- Well-designed clinical trials showing that inhibition of MGMT is clinically beneficial for patients with gliomas are lacking.

Open questions

- Does MGMT as a target hold promise for the treatment of TMZ-resistant gliomas?
- Is increasing TMZ dose intensity by extended administration actually a beneficial strategy for inactivating MGMT?
- Is MGMT promoter methylation a favorable biomarker for glioblastoma?
- How to overcome dose-limiting myelosuppressive toxicity of TMZ while maintaining its efficacy?
- How to prevent chemoresistance to TMZ and reduce the doses of TMZ and thus its toxicities without compromise of its efficacy?

Alkylating agents such as TMZ are the principle first-line chemotherapeutic agents used for the treatment of recurrent high-grade glioma and have also been used to treat advanced malignant melanoma and other solid neoplasias.\(^1,2\) Little progress has been made in the treatment of metastatic glioma...
and melanoma, mainly due to cancer cell resistance to chemotherapy. A variety of mechanisms account for cancer cell resistance to chemotherapy, which include decreased drug uptake into the cell, increased drug efflux, intracellular drug inactivation, repair of drug-induced damage, or resistance to drug-induced apoptosis. 3, 4 TMZ is a prodrug that undergoes spontaneous decomposition in solution at physiological pH to the reactive intermediate 5-(3-methyl-1-triazeno)imidazole-4-carboxamide, which methylates the N7 and O6 positions of guanine and the N3 position of adenine. 5 The methyl adducts, O6-meG, N7-methylguanine (N7-meG), and N3-methyladenine (N3-meA) result in a continuous cycle of DNA base mismatch repair (MMR) with eventual strand breaks, ultimately leading to cellular apoptosis (Figure 1). 6 Therefore, TMZ exerts its chemotherapeutic efficacy for cancer by inducing apoptosis of cancer cells. It has been demonstrated that TMZ-triggered apoptosis requires Fas/CD95/Apo-1 receptor activation in glioma cells with wild-type p53, whereas the same DNA lesion triggers the mitochondrial apoptotic pathway in p53-mutated glioma. 7

However, acquired chemoresistance to TMZ developed in cancer cells has been shown to be a major limitation to this therapy, as >90% of recurrent gliomas show no response to a second cycle of chemotherapy. 8 Several mechanisms are believed to have critical roles in the chemoresistance to TMZ. The primary mechanism involves MGMT, which is a small enzymatic protein acting to directly remove O6-meG from the O6-guanine position. 9 It has been demonstrated that MGMT repairs the principle O6-meG-alkylation site of TMZ, limiting its effects. High levels of MGMT are reported in the brain and other tumors and correlate with resistance to TMZ. 10 Interestingly, DNA MMR and poly(ADP-ribose) polymerase (PARP) may also contribute to chemoresistance. 11 The anti-tumor effect of TMZ is resulted from the induction of methyl adducts at several DNA bases: O6-guanine, N7-guanine, and N3-adenine. The base excision repair (BER) pathway is important in the repair of DNA damage at the N7-guanine and N3-adenine adducts, and high levels of BER activity can confer resistance to TMZ, similar to MGMT. The MMR system, which is critical for the removal of DNA replication errors leads to reiterative attempts to repair the damage, or a ‘futile cycle’ of DNA repair, ultimately leading to apoptosis. 12

Previous studies have shown that inhibition of MGMT increases TMZ sensitivity only in MMR-proficient cells but not in MMR-deficient cells. 13 Therefore, it is conceivable to consider MGMT as a potential therapeutic target for the treatment of cancers that are potentially resistant to TMZ.

In this review article, we will primarily present the rationale for combining inhibitors of MGMT with TMZ to improve the efficacy of treatment for neurological tumors, particularly gliomas. Moreover, the potential underlying mechanisms for resistance to TMZ chemotherapy will be discussed, with highlight of the important role of the DNA damage response pathways.

**TMZ induces DNA damage and cell apoptosis through O6-meG-mediated MMR**

Alkylating agents can generate up to 13 adducts in DNA. Although the minor product O6-meG accounts for <8% of total alkylation, it exerts the greatest potential for apoptosis. 14 O6-meG is processed into DNA double-strand breaks (DSBs) in a DNA MMR-dependent manner, requiring two rounds of DNA replication (Figure 2). 15, 16 These DSBs may then trigger apoptotic cell death in glioblastoma multiforme (GBM). 7 O6-meG is the best example of how a defined DNA lesion triggers the DNA damage response. Although this small DNA adduct does not block DNA replication, it occurs in MGMT lacking cancer cells and may be responsible for the majority of mutations, recombination, clastogenicity, and apoptotic effects of methylating agents that have the ability to methylate oxygen in DNA. 17 One of the key transducing kinases of the
DNA replication checkpoint is the ataxia telangiectasia and Rad3-related kinase (ATR). ATR localizes to sites of stalled DNA replication forks, which is mediated by the ATR-interacting protein (ATRIP), a prerequisite for ATR.\textsuperscript{18} Pairing (or mismatch) of O\textsuperscript{6}-meG with thymine (i.e., O\textsuperscript{6}-meG/T) is a substrate of MutS\textsubscript{a}/MutL\textsubscript{a}-dependent MMR.\textsuperscript{19} It has been observed that MutS\textsubscript{a} bound to O\textsuperscript{6}-meG/T stimulates phosphorylation of ATR/ATRIP and Chk1, and thus binding of MutS\textsubscript{a} to O\textsuperscript{6}-meG/T lesions may be sufficient to activate the DNA damage response.\textsuperscript{20} It is also conceivable that O\textsuperscript{6}-meG/T mismatches directly lead to DSBs due to nuclease attack at single-stranded DNA (ssDNA) arising from the futile MMR process. Cells with O\textsuperscript{6}-meG/T lesions have to pass through a second cell cycle where MMR processing the lesions leads to secondary DNA lesions, which interfere with DNA replication. Consequently, replication blockade and DSBs occur as a result of stalled replication forks and fork collapse.\textsuperscript{16}

Following alkylation stress, in the absence of MGMT, the repair proteins are required and are to be activated for recombination repair. Many other pathways are also involved in repairing DNA damage induced by alkylating agents, which include homologous recombination (HR), non-homologous end-joining (NHEJ), BER, polymerase bypass, and MMR.\textsuperscript{21} Double-stranded DNA break (DSB) is a dangerous DNA lesion and if left unrepaired result in severe genomic instability. The two major pathways for repair of DSBs are HR and NHEJ, BER, polymerase bypass, and MMR.\textsuperscript{21}

Figure 2  MGMT and other DNA repair mechanisms deal with DNA damage produced by the alkylating agent TMZ in cancer cells. TMZ cause potentially cytotoxic DNA lesions such as O\textsuperscript{6}-meG (red circle), N\textsuperscript{7}-meG (red ellipse) and N\textsuperscript{3}-meA (red ellipse). (a) MGMT removes the O\textsuperscript{6}-alkylguanine DNA adduct, O\textsuperscript{6}-meG, through covalent transfer of the alkyl group to the conserved active-site cysteine and restores guanine to normal. After receiving a methyl-group from O\textsuperscript{6}-meG, MGMT is inactivated and subjected to ubiquitin-mediated degradation. (b) If an O\textsuperscript{6}-meG DNA adduct escapes MGMT repair, it would form a base pair with thymine during DNA replication. The mismatched base pair of the persistent O\textsuperscript{6}-meG with thymine is recognized by the MMR pathway, resulting in futile cycles of repair leading to DSBs and triggering apoptosis. (c) N\textsuperscript{7}-meG and N\textsuperscript{3}-meA DNA adducts are efficiently repaired by the BER pathway and normally contribute little to TMZ cytotoxicity in cancer cells. Methoxyamine binds to apurinic/apyrimidinic (AP) DNA damage sites produced by methylpurine glycosylase (MPG, blue circle), the first step in BER processing. Methoxyamine-bound AP sites are refractory to AP endonuclease cleavage, resulting in the blockage of the BER pathway, leading to strand breaks, disrupted replication, and increased cytotoxicity of TMZ. Chemistry of the lesion and the repair intermediates throughout the repair process are highlighted as the three major steps for BER: lesion recognition/strand scission, gap tailoring, and DNA synthesis/ligation.
sequence homology to perform an error-free break correction that preserves the original DNA sequence. The central reaction of the HR pathway, namely the homology search and strand invasion, is performed by Rad51-coated 3' ssDNA tails generated by DNA end resection of the break.23,24 The formation of this nucleoprotein filament at ssDNA is promoted and stabilized by BRCA2.25,26 Both Rad51 and BRCA2 are essential for HR in mammalian cells, although the functions of Rad51 and BRCA2 in other repair pathways have not been elucidated.

MGMT activity promotes resistance to TMZ

Overexpression of MGMT prevents cancer cells from death induced by alkylating agents with a correlation between MGMT activity and tumor drug resistance.9 MGMT removes the O6-alkylguanine DNA adduct through covalent transfer of the alkyl group to the conserved active site, cysteine, and restores the guanine to normal.27 After receiving a methyl-group from O6-meG, MGMT is inactivated and subjected to ubiquitin-mediated degradation. A similar suicidal enzyme reaction occurs when MGMT transfers and accepts an alkyl group from O6-benzylguanine (O6-BG) or O6-(4-bromothenyl) guanine (PaTrin-2).

As one molecule of MGMT removes only one alkyl molecule, an excess of DNA adducts at the O6-position could completely deplete MGMT. The stoichiometric and irreversible transfer of adducts to internal cysteine residues at position 145 prevents GC→AT transitions or cross-linking induced by mono-functional and bi-functional alkylating agents, respectively. O6-meG adducts, if unrepaird, cause O6-meG:C→O6-meG/T transition mutations following DNA replication. The MMR complexes (MutSα and MutLα) have an affinity for O6-meG/T mismatches.28 These mismatches are initially detected and processed by MutS and MutL homologs (MSH and MLH, respectively), which then signal to activate the ataxia telangiectasia-mutated kinase checkpoint kinase 2 (Chk2) and ATR-Chk1 pathways. This leads to the activation of a transient checkpoint in the second S phase and eventual cell cycle arrest at the G2 phase.29

MGMT-mediated repair is unique among DNA repair pathways. First, it acts alone without relying on any other proteins or cofactors. It transfers the alkyl group to an internal cysteine residue in the protein, acting as both a transferase and an acceptor of the alkyl group. It inactivates itself after receiving the alkyl group from guanine, and thus is a suicidal protein. Finally, it repairs DNA lesions in a stoichiometric fashion. Thus, investigation of potential drug interactions that modulate MGMT could help improve the efficacy of chemotherapies utilizing alkylating agents.30

Inhibition of MGMT enhances TMZ-induced cytotoxicity in cancer cells

A number of O6-guanine derivatives and related compounds have been used to test whether they can bind and inactivate MGMT in a variety of ways in preclinical and clinical settings (Figure 3).

O6-BG and PaTrin-2. One of the potent agents is O6-BG, which is so far the most extensively studied direct MGMT inhibitor.31 Another potent agent is PaTrin-2, a pseudosubstrate inactivator of MGMT, which was reported to be more potent than O6-BG in inactivating recombinant MGMT protein.32 O6-BG and PaTrin-2 have been shown to reverse the therapeutic resistance to TMZ by modulating MGMT expression in a variety of human tumor cell lines and xenograft models that include melanoma, brain, prostate, and colon cancers.33,34

However, many clinical trials with O6-BG or PaTrin-2 have observed an increase in toxicity or adverse events, resulting in a significant dose reduction of TMZ. This might explain why, in any of the reported clinical trials, the outcome has not been improved by the inclusion of O6-BG or PaTrin-2. In addition, in all the studies, myelosuppression manifested as neutropenia, leukopenia, and thrombocytopenia was mainly responsible for dose limitation. A recent study evaluated a combination of TMZ and O6-BG in patients with anaplastic glioma: only 3% of the former and 16% of the latter responded to therapy, and overall, grade 4 hematological events were observed in 48% of the patients.35 In another phase II trial, 104 patients with metastatic melanoma were treated with TMZ alone or a combination of PaTrin-2 and TMZ on days 1–5 every 28 days for up to 6 cycles.36 Combination with PaTrin-2 did not significantly influence the overall response rate (13.5 versus 17.3%) and the median time to disease progression (65.5 versus 68.0 days).

Resveratrol. Resveratrol is a naturally occurring compound that possesses anti-cancer effects.37,38 Resveratrol has been reported to reverse TMZ resistance by downregulation of MGMT in T98G glioblastoma cells through a NF-κB-dependent pathway.39 Combination with TMZ and
resveratrol decreases the 50% inhibiting concentration of TMZ and increases apoptosis in TMZ-resistant T98G cells. Lin et al.\(^46\) indicated that TMZ-induced ROS/ERK-mediated autophagy protected glioma cells from apoptosis, and combined treatment with resveratrol and TMZ improved the efficacy of chemotherapy for brain tumors. Yuan et al.\(^41\) demonstrated that resveratrol enhanced TMZ-mediated anti-tumor effects in GBM via a ROS-dependent AMPK-TSC-mTOR signaling pathway. Therefore, resveratrol is a promising agent to overcome resistance to TMZ, and its effect on MGMT is one of the possible mechanisms.

**Oncolytic viruses.** Oncolytic viruses, such as adenoviruses and herpes simplex viruses (HSV), have been introduced into clinical trials as a new treatment for malignancies.\(^45\) During viral infection, the extraordinary amount of exogenous viral DNA mimics the damaged DNA with DSBs that are processed by the cellular DNA-repair machinery. Unlike the DNA-damaging agents, viruses inactivate or manipulate the cellular DNA-repair system for the viruses' benefit.\(^43\)

Adenovirus E1A protein has been shown to efficiently inhibit the promoter of MGMT and thus may reduce the chemoresistance of cancer cells to TMZ.\(^44\) A recent study revealed synergistic tumor cell killing by the combination of TMZ with oncolytic viruses, A-24-RGD.\(^44\) The adenovirus E1A protein binds with p300 protein to inhibit the transcription of MGMT, leading to silencing of the MGMT promoter. It has been reported that combination of TMZ with a mutant HSV, G207, produced a synergistic effect on globlastoma cell killing *in vitro* and *in vivo*, and the synergism depended on the activation of DNA repair pathways.\(^45\) A recent study showed that the combination of G47-Delta and TMZ acted synergistically in killing glioblastoma stem cells through oncolytic HSV-mediated manipulation of DNA damage responses, indicating that this strategy is highly efficacious in representative preclinical models and warrants clinical translation.\(^36\) As combination of TMZ and an agent with a different mode of action makes the emergence of resistance to TMZ in cancer cells less likely, treatment with oncolytic viruses and DNA-damaging agents, such as TMZ, may result in a better therapeutic effect in gliomas.

**Quinolone derivatives.** Quinolone derivatives such as 2-phenyl-4-quinolones can induce cytotoxicity in many human cancer cell lines.\(^47,48\) It has been shown that a novel synthetic quinolone, 20-fluoro-6, 7-methylenedioxy-2-phenyl-4-quinolone (CHM-1) inhibits the growth of hepatocellular carcinoma *in vitro* and *in vivo*.\(^48\) CHM-1 also induces DNA damage in human osteogenic sarcoma cells and murine colorectal adenocarcinoma cells.\(^49,50\) Tipifarnib is synthesized by the condensation of the anion of 1-methylimidazole with a 6-(4-chlorobenzoyl) quinolone derivative.\(^51\) In a phase I trial, tipifarnib was well tolerated at 300 mg bid given discontinuously (days 21/28) in 4-week cycles, concurrently with standard chemo/radiotherapy.\(^52\) An ongoing phase II study should evaluate the efficacy of tipifarnib with TMZ in patients with newly diagnosed glioblastoma and not receiving enzyme-inducing anti-epileptic drugs.

**S-adenosylmethionine and S-adenosylhomocysteine.** Methylation of DNA and mRNA is controlled by levels and ratios of the intracellular S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy). A previous study showed that experimentally elevated AdoHcy levels significantly decreased MGMT mRNA levels by >50% in all MGMT-expressing cancer cell lines, which is most likely the result of impaired mRNA methylation.\(^53\) Thus, inhibition of MGMT expression by alterations in AdoMet/AdoHcy ratio could be used as a novel pharmacological strategy to improve the responsiveness to TMZ and other alkylating agents.

**Inhibition of PARP and BER pathway may be alternative strategies to enhance TMZ activity**

Poly(ADP-ribosyl)ation is a ubiquitous protein modification found in mammalian cells that modulates many cellular responses, including DNA repair.\(^54\) An alternative strategy to enhance TMZ activity is to inhibit PARP, mainly PARP-1 and PARP-2 (Figure 4).\(^55\) It is hypothesized that PARP inhibition is able to restore TMZ activity due to a change in the cytotoxicity locus of TMZ from O\(^{6}\)-meG to N\(^{3}\)-meA.\(^56\) Majority of TMZ-induced lesions (N\(^{7}\)-meG and N\(^{3}\)-meA) are substrates of the BER pathway, and thus the BER pathway has emerged as an attractive target for reversing TMZ resistance (Figure 4).\(^56\) Inhibition of BER pathway leads to the accumulation of repair intermediates that induce energy depletion–mediated cell death, and TMZ-induced cell death via BER inhibition is dependent on the availability of nicotinamide adenine dinucleotide (NAD \(^+\)). It has been shown that inhibition of both BER and NAD \(^+\) biosynthesis significantly sensitizes glioma cells with elevated expression of MGMT.\(^56\) Therefore, dual targeting these two interacting pathways (BER and NAD \(^+\) biosynthesis) may be an effective treatment for recurrent and resistant GBM.

**Increasing TMZ dose intensity by extended administration: a beneficial strategy for inactivating MGMT?**

As a suicide enzyme, MGMT is inactivated following each reaction. Therefore, theoretically, if the rate of DNA alkylation outpaces the rate of MGMT synthesis, the enzyme would be depleted. This property leads to the exploration of extended dose-dense TMZ regimens, with the idea that they could potentially deplete MGMT in tumor cells by overwhelming the cells' ability to synthesize MGMT, which might enhance therapeutic activity.\(^57\)

The concept of MGMT depletion was validated by Tolcher *et al.*,\(^58\) who showed that MGMT enzyme activity was depleted in peripheral blood mononuclear cells during treatment with TMZ for either 7 consecutive days every 14 days (7 of 14-day regimen) or 21 consecutive days every 28 days (21 of 28-day regimen).

The originally approved TMZ dosing regimen is 150–200 mg/m\(^2\) per day (days 1–5 every 28-day cycle (5 of 28 days)). However, extended dosing regimens (i.e., 7 of 14 days, 21 of 28 days, 6 of 8 weeks, or continuously daily) allow for administration of a higher cumulative dose per cycle and have been...
shown to achieve a prolonged MGMT depletion and higher levels of TMZ at the site of the central nervous system, which may enhance cytotoxic activity.  

Phase I trials have shown that such a strategy effectively depletes MGMT levels while permitting an almost two-fold greater level of exposure to the drug with minimal additional toxicity. It has been reported that extended administration of low-dose TMZ sensitizes leukemic blasts to conventional doses of TMZ in TMZ-refractory patients, and MGMT activity can be reduced by approximately 80% in patients treated with this dosing schedule. These could be due to the fact that extended administration of TMZ, even at relatively low daily doses, leads to significant and prolonged depletion of MGMT activity, which may allow the patients to tolerate the increased systemic adverse events and enhance the anti-tumor activity of the agent. Despite these encouraging findings, two recent randomized controlled trials failed to produce overall survival benefit of increasing TMZ dose intensity by extended administration in recurrent and newly diagnosed gliomas, which casts concerns on the usefulness of this strategy.

**MGMT promoter methylation is a favorable biomarker for glioblastoma**

MGMT methylation status, an indicator of gene silencing, emerges as a potentially important molecular test to determine which patients with glioblastoma will benefit and should receive TMZ. The MGMT promoter is typically reported methylated in 30–60% of glioblastomas and in 30–90% of low-grade gliomas.

Several trials have shown that MGMT promoter methylation correlates with better outcome of therapy. Wick et al. reported that TMZ chemotherapy produced no benefit in patients without MGMT promoter methylation in elderly patients with anaplastic astrocytoma or glioblastoma. Malmström et al. showed that patients treated with TMZ who had MGMT promoter methylation had significantly longer OS than those without (9.7 versus 6.8 months). Similarly, Lechapt-Zalcman et al. also showed that patients with glioblastomas who harbored MGMT methylation had a significantly longer OS compared with patients who had wild-type MGMT (21.7 versus 15.1 months; P = 0.025). In addition, it has been shown that treatment with radiotherapy plus chemotherapy or chemotherapy alone, compared with radiotherapy alone, achieves longer PFS for patients with MGMT methylation but not in those without. Low MGMT expression and MGMT promoter methylation are both predictive markers for slower tumor progression in patients with glioblastoma. Thus, MGMT promoter methylation may be a useful biomarker to stratify glioblastoma patients whether or not to receive treatment with alkylating agents. However, not all clinical trials have demonstrated the association of MGMT methylation and better responses. Therefore, well-designed clinical trials are required to provide convincing evidence showing that inactivation of MGMT is clinically beneficial.

**Conclusions**

TMZ, an alkylating agent, has a prominent role in gliomas by inducing DNA damage and cell apoptosis. However, resistance to TMZ emerges with prolonged treatment, mainly due to MGMT, which repairs O6-meG lesion, which posts a major therapeutic challenge. Thus, inhibition of MGMT with several O6-guanine derivatives and related compounds have been explored and shown to enhance TMZ-induced cytotoxicity in cancer cells. In addition, MGMT promoter methylation is a favorable biomarker for glioblastoma. Currently, there is a challenge in overcoming dose-limiting myelosuppressive toxicity of TMZ while maintaining its efficacy. Well-designed clinical trials are required to provide convincing evidence showing that inhibition of MGMT is clinically beneficial for patients with gliomas by preventing chemoresistance to TMZ and reducing the doses of TMZ and thus its toxicities without compromise of its efficacy. It is, however, anticipated that, with the availability of tumor-targeting strategies, MGMT as a target holds promise for the treatment of TMZ-resistant gliomas.
Conflict of Interest
The authors declare no conflict of interest.

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1. Bei R, Marzocchella L, Turnici M. The use of temozolomide for the treatment of malignant tumors: clinical evidence and molecular mechanisms of action. Recent Pat Anticancer Drug Discov 2010; 5: 172–187.
2. Tinri VA, Patel SP, Hwu WJ. The safety of temozolomide in the treatment of malignancies. Expert Opin Drug Saf 2009; 8: 493–499.
3. O’Brien V, Brown R. Signaling cell cycle arrest and cell death through the MMR System. Carcinogenesis 2006; 27: 682–692.
4. Ferlin MG, Chiarelotto G, Gasparotto V, Dalla Via L, Pezzi V, Barzon L. A promising target for the treatment of gliomas: the novel quinolone CHM-1. Cell Death and Disease 2010; 11: 1407–1415.
5. Jiang H, Lin H, Zhang X, Li J. Revertase reverses temozolomide resistance by downregulation of MGMT in T98G glioblastoma cells by the NF-kappaB-dependent pathway. Oncol Rep 2012; 27: 2050–2056.
6. Liu J, Doty T, Gibson B, Heyer WD. Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. Nat Struct Mol Biol 2010; 17: 1260–1262.
7. Wang SW, Pan SL, Huang YC, Guh JH, Chiang PC, Huang DY. The synthesized 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (CHM-1) promoted G2/M arrest through inhibition of CDK1 and induced apoptosis through the mitochondrial-dependent pathway.
pathway in CT-26 murine colorectal adenocarcinoma cells. J Gastroenterol 2009; 44: 1055–1063.
51. Thomas X, Elhami M. Tiotifarnib in the treatment of acute myeloid leukemia. Biologics Targets Ther 2007; 1: 415–424.
52. Ngheimphu PL, Wen PY, Lamborn KR, Drappatz J, Robins HI, Fink K et al. A phase I trial of tiotifarnib with radiation therapy, with and without temozolomide, for patients with newly diagnosed glioblastoma. Int J Radiat Oncol Biol Phys 2011; 81: 1422–1427.
53. Hermes M, Geisler H, Osswald H, Riehle R, Kloos D. Alterations in S-adenosylhomocysteine metabolism decrease O6-methylguanine DNA methyltransferase gene expression without affecting promoter methylation. Biochem Pharmacol 2008; 76: 2100–2111.
54. Chalmers AJ. The potential role and application of PARP inhibitors in cancer treatment. Br Med Bull 2009; 89: 23–40.
55. Cheng CL, Johnson SP, Keir ST, Quinn JA, Ali-Osman F, Szabo C et al. Poly(ADP-ribose) polymerase-1 inhibition reverses temozolomide resistance in a DNA mismatch repair-deficient malignant glioma xenograft. Mol Cancer Ther 2005; 4: 1364–1368.
56. Goeller EM, Gimmie B, Brown AR, Lin YC, Wang XH, Sugrue KF et al. Overcoming temozolomide resistance in glioblastoma via dual inhibition of NAD+ biosynthesis and base excision repair. Cancer Res 2011; 71: 2308–2317.
57. Wick W, Platten M, Weiler M. New (alternative) temozolomide regimens for the treatment of glioma. Neuro-oncol 2009; 11: 69–79.
58. Tochner AW, Gerson SL, Denis L, Geyer C, Hammond LA, Patnaik A et al. Marked inactivation of O6-ethylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. Br J Cancer 2003; 88: 1004–1011.
59. Neyna B, Tosoni A, Hwu WJ, Reardon DA. Dose-dense temozolomide regimens: antitumor activity, toxicity, and immunomodulatory effects. Cancer 2010; 116: 2666–2677.
60. Tawbi HA, Villaruz L, Farhini A, Moschos S, Sulecki M, Viverette F et al. Inhibition of DNA repair with MGMT pseudosubstrates: phase I study of temozolomide in combination with dacarbazine in patients with advanced melanoma and other solid tumours. Br J Cancer 2011; 105: 773–777.
61. Mederos BC, Kohri HE, Gottlib J, Coutre SE, Zhang B, Arber DA et al. Tailored temozolomide therapy according to MGMT methylation status for elderly patients with acute myeloid leukemia. Am J Hematol 2012; 87: 45–50.
62. Brada M, Stening S, Gabe R, Thompson LC, Levy D, Rampling R et al. Temozolomide versus procarbazine, lonidine, and vincristine in recurrent high-grade glioma. J Clin Oncol 2010; 28: 4601–4608.
63. Gilbert MR, Wang M, Ailade KD, Stupp R, Hegi M, Jaedicke KA et al. RTOG 0525: A randomized phase III trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM). J Clin Oncol 2011; 29: (suppl; abstr 2006).
64. Weiler M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol 2010; 6: 39–51.
65. Havik AB, Brandal P, Honne H, Dahlback HS, Scheie D, Heitkon M et al. MGMT promoter methylation in gliomas-assessment by pyrosequencing and quantitative methylation-specific PCR. J Transl Med 2012; 10: 38.
66. Everhard S, Kaloshi G, Cirinère A, Benouaich-Amiel A, Lejeune J, Marie Y et al. MGMT methylation: a marker of response to temozolomide in low-grade gliomas. Ann Neurol 2006; 60: 740–743.
67. Christmann M, Nagel G, Horn S, Krahm U, Wieser W, Sommer C et al. MGMT activity, promoter methylation and immunohistochemistry of pretreatment and recurrent malignant gliomas: a comparative study on astrocytoma and glioblastoma. Int J Cancer 2010; 127: 2106–2118.
68. Wick W, Stupp R, Melsner C, Fleisch J, Tabatabai G, Schulz H et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. Lancet Oncol 2012; 13: 707–715.
69. Malmström A, Granberg BH, Marosi C, Stupp R, Spazaz D, Schulz H et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 65 years with glioblastoma: the Nordic randomised, phase 3 trial. Lancet Oncol 2012; 13: 916–926.
70. Lechapt-Zalcman E, Levallet G, Dugue AE, Vital A, Diebold M, Menel P et al. O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation and low MGMT-encoded protein expression as prognostic markers in glioblastoma patients treated with biodegradable carmustine wafer implants after initial surgery followed by radiotherapy with concomitant and adjuvant temozolomide. Cancer 2012; 118: 4544–4554.
71. Reifenberger G, Hentschel B, Felsberg J, Schackert G, Simon M, Schnell O et al. Predictive impact of MGMT promoter methylation in glioblastoma of the elderly. Int J Cancer 2012; 131: 1342–1350.
72. Sonoda Y, Yokosawa M, Saito R, Kanamori M, Yamashita Y, Kumabe T et al. O6-Methylguanine-DNA methyltransferase determined by promoter hypermethylation and immunohistochemical expression is correlated with progression-free survival in patients with glioblastoma. Int J Clin Oncol 2010; 15: 352–358.
73. Chinol OL, Bari`e M, Fuentes S, Eudes N, Lancelet S, Metellus P et al. Correlation between O6-methylguanine-DNA methyltransferase and survival in inoperable newly diagnosed glioblastoma patients treated with neoadjuvant temozolomide. J Clin Oncol 2007; 25: 1470–1475.
74. Preussner M, Charles Janzer R, Felsberg J, Reifenberger G, Hamou MF, Dei`ersen AC et al. Anti-O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation and low MGMT-expression as predictors for glioblastoma multiforme: observer variability and lack of association with patient survival impede its use as clinical biomarker. Brain Pathol 2008; 18: 520–532.
75. Hassel JC, Sucker A, Eider L, Kurzen H, Moi I, Stroesemann C et al. MGMT gene promoter methylation correlates with tolerance of temozolomide treatment in melanoma but not with clinical outcome. Br J Cancer 2010; 103: 820–826.