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MINI REVIEW

Temozolomide in malignant gliomas: current use and future targets

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Abstract Temozolomide (TMZ) is an oral alkylating agent that is regarded as a tolerable and effective drug. When combined with radiotherapy in patients with newly diagnosed glioblastoma, survival is significantly prolonged. This finding has led to widespread use of TMZ for patients with this disease. We summarize developing concerns regarding the use of TMZ, imaging of malignant gliomas, and the pharmacology of TMZ—mechanism of action, scheduling and strategies for overcoming resistance.

Keywords Temozolomide · Brain tumor · Alkylating chemotherapy · Malignant glioma · Glioblastoma · Tumor resistance

Introduction

Temozolomide (TMZ) provided hope for patients with primary brain tumors and their physicians when the drug was approved by the FDA in 1999. Subsequently, the European Organization for Research and Treatment of Cancer (EORTC) 22981/26981-National Cancer Institute of Canada (NCIC) CE.3 trial [1, 2] demonstrated an improved median survival, representing the first such improvement since that seen with radiation therapy (RT) in the mid-1970s. TMZ received FDA approval for refractory anaplastic astrocytoma (AA), followed by a first-line indication for glioblastoma (GBM). The EORTC-NCIC or Stupp regimen [1] is now the standard of care for patients with malignant gliomas. We review some emerging clinical issues with the use of TMZ, as well as new developments in TMZ modulation.

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Overview of astrocytomas and the role of TMZ

Malignant or high-grade astrocytomas are glial derived highly infiltrating tumors that are WHO grade III (AA) or WHO grade IV (GBM) [3]. Three quarters of all gliomas are astrocytomas, and malignant astrocytomas represent the major cause of death from primary brain tumors [3]. While it is recognized that more complete resections lead to better outcomes, surgical resection alone is inadequate for cure because of the infiltrative nature of these tumors. Radiotherapy is central in the treatment of high-grade gliomas, and leads to improved survival. Limited field fractionated radiation is generally employed, covering the enhanced lesion as well as peri-tumoral edema; the standard dose is 60 Gray (Gy). Historically, the role of systemic chemotherapy was not well established, with no single study demonstrating a survival advantage, and only meta-analyses showing benefit [4, 5]. Local therapy with impregnated carmustine wafers [polifeprosan 20 with carmustine implant (Gliadel® wafer), MGI Pharma Inc.] in addition to surgery or radiation has demonstrated a survival advantage in patients with newly diagnosed high-grade gliomas [6]. The wafer is FDA approved for use in both newly diagnosed malignant gliomas, including AA and GBM, and in recurrent GBM in patients undergoing resective surgery [7].

In 2002 and 2005, Stupp et al. [1, 8], published the results of phase II and phase III trials, respectively, that employed TMZ with concurrent radiotherapy followed by TMZ monotherapy in patients with newly diagnosed GBM. Previously, Yung et al. [9, 10] had demonstrated the benefit of TMZ in patients with WHO grade III astrocytomas and in patients with GBM in first relapse. The randomized EORTC-NCIC phase III study documented a median survival of 14.6 months with RT plus TMZ compared with 12.1 months for RT alone; 2- and 5-year survival were also improved. These results led to the EORTC-NCIC regimen as the international standard. Thus, TMZ is generally regarded by oncologists as tolerable, effective, and easy to administer.

Treatment factors in malignant gliomas

The recursive partitioning analysis (RPA) classification developed by Curran et al. [11], stratifies patients based on pre-treatment factors including age, functional status, neurologic status, and extent of surgery. This classification has long been used and is more predictive of outcome than the type of adjuvant treatment. In the EORTC-NCIC study, patients younger than 50 years of age and with a Karnofsky performance status (KPS) of 90–100 (class III) had a 43% chance of surviving 2 years versus the least favorable group (class V; age 50 or older and having a Mini-Mental Status Examination score of <27, and KPS <70) having only a 16.5% chance of 2-year survival [12]. The benefit of receiving concurrent TMZ and RT versus RT alone was most evident in the favorable RPA class (class III) with a gain of 7 months in median survival while the least favorable RPA group (class V) had a modest gain of 1.1 months.

Methylation of the promoter region of the *MGMT* gene in the tumor specimen is associated with superior outcomes in patients treated with alkylators [13]. Hegi et al. [14], described *MGMT* promoter methylation in 45% of 206 specimens that were assessable from the EORTC-NCIC trial. Patients whose tumors contained a methylated *MGMT* promoter experienced the greatest survival benefit (median 21.7 vs. 15.3 months).

Treatment of the elderly (age >70 years) has often consisted of short courses of RT [15]. An alternative option described in the literature is TMZ monotherapy without RT [16, 17]. The upper age limit in the EORTC-NCIC study was 70 years, but Kimple et al., recently demonstrated that the EORTC-NCIC regimen in good performance status elderly patients with GBM can be tolerated [18]. The median survival was 38 weeks in patients treated with the EORTC-NCIC regimen, as compared to 32 weeks with short course radiation and 23.1 weeks with high dose radiation without chemotherapy.

Temozolomide pharmacokinetics and mechanism of action

Temozolomide is well absorbed after oral administration [19]. Food has little effect on absorption. The average volume of distribution is reported to be 17 l/m^2, and the half-life of the parent drug is approximately 1.8 h [19, 20]. TMZ CSF concentrations reach 30–40% of plasma concentrations. This fact alone cannot account for the significant activity of TMZ in brain tumors. While treatment of neoplastic meningitis is enhanced by the achievement of 'therapeutic' CSF concentrations of chemotherapeutic agents, CSF concentrations may not accurately reflect delivery of drugs to tumors involving brain parenchyma. Drug delivery includes, in addition to penetration through the blood–brain barrier, delivery to tumor cells and peri-tumoral cells [21]. The contribution of parent drug versus metabolite must also be considered. For many drugs, including TMZ, the specifics of drug distribution in the CNS of patients with tumors are not known.

Preclinical studies demonstrated variable efficacy of TMZ with different schedules of administration [20, 22]. A daily for 5 days schedule was shown to have greater activity than single doses. The most widely used dose and schedule has thus been 150 or 200 mg/m^2 daily for 5 days, repeated every 28 days. When used concomitantly with radiation, the recommended dose of TMZ is 75 mg/m^2 per day. No dosage adjustments are required for mild to moderate renal or hepatic impairment. No guidelines exist for dosage adjustments for severe renal or hepatic impairment; it would appear that such adjustments are not required.

Following oral absorption, TMZ is hydrolyzed in aqueous solution to methyl-triazeno-imidazole-carboxamide (MTIC), also the active metabolite of dacarbazine (Fig. 1). But unlike dacarbazine, a prodrug whose conversion to MTIC requires initial metabolism, TMZ conversion to MTIC is spontaneous. MTIC is rapidly converted to the inactive 5-aminoimidazole-4-carboxamide (AIC) and to the electrophilic alkylating methyldiazonium cation that transfers a methyl group to DNA. The DNA-methyl adducts are responsible for cytotoxicity. Alkylation of the O^6 position of guanine accounts for only about 5% of DNA adducts, but is primarily responsible for the cytotoxic effects of TMZ. The N^7 of guanine and the N^3 of adenine represent the majority of DNA-methyl adducts [20]. The O^6-methylguanine (O^6-meG) lesion leads to DNA double strand breaks and subsequent cell death via apoptosis and/or autophagy (Fig. 2) [23–25].

MGMT repairs the O^6-meG lesion, and thus high levels of MGMT are thought to contribute to resistance to TMZ [14, 22]. Conversely, methylation of the promoter of the *MGMT* gene silences the gene, and would be expected to enhance the cytotoxicity of O^6-meG lesions. Variable

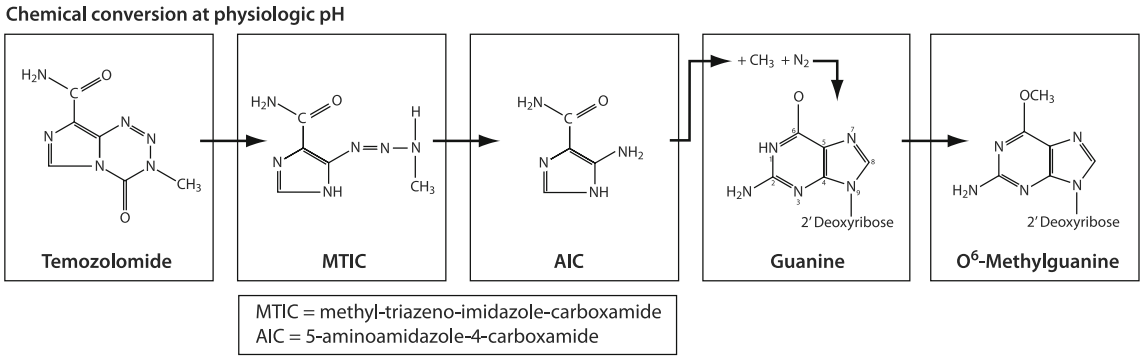

Fig. 1 Chemical structure of temozolomide and metabolites

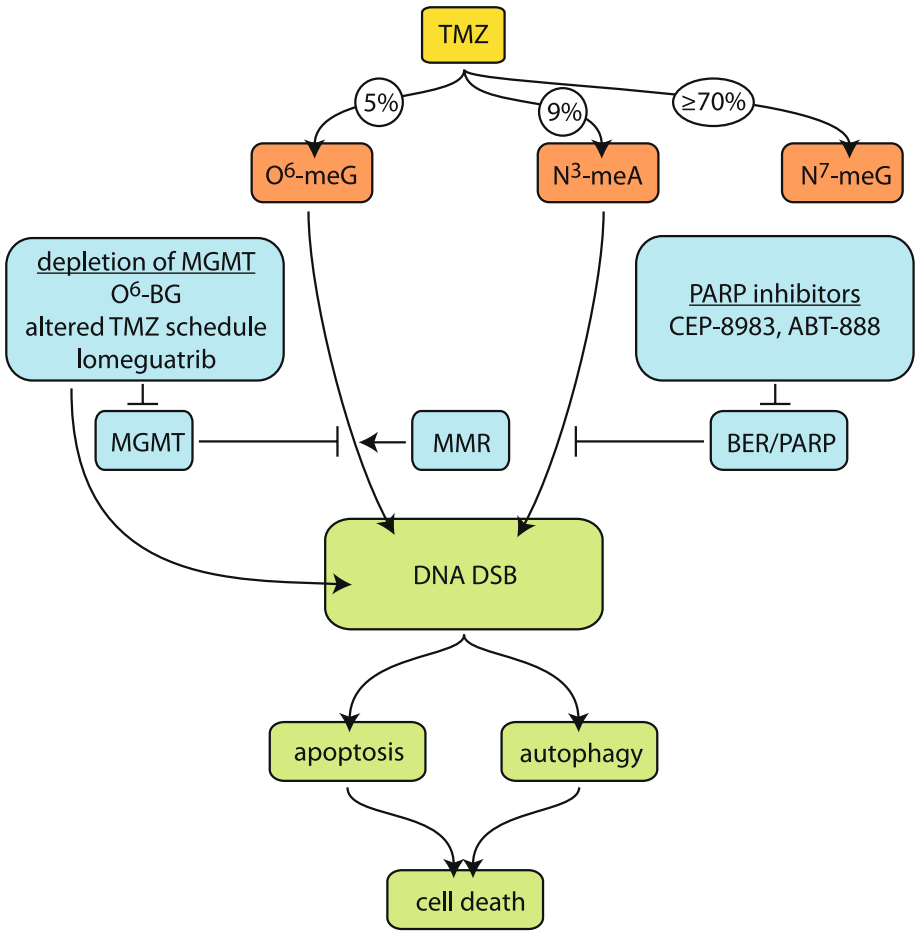

Fig. 2 Temozolomide: site of action and targets for regulation with corresponding agents. O^6-meG DNA adducts account for only 5% of TMZ-induced DNA lesions, but they are responsible for the cytotoxic effect. MGMT repairs the lesions, resulting in resistance to TMZ. When MGMT is depleted (such as by O^6-BG or varied dosing schedules) or suppressed by methylation of the gene promoter, cytotoxicity of TMZ is enhanced. MMR recognizes the abnormal base pairs containing O^6-meG, eventually leading to DNA DSB and cell death. N^3-meA adducts are also cytotoxic, but are usually repaired by the BER system. Pharmacologic inhibition of PARP inhibits the functioning of the BER system, potentially enhancing TMZ cytotoxicity

TMZ	=	temozolomide	O^6-BG	=	O^6-benzylguanine
O^6-meG	=	O^6-methylguanine	MMR	=	mismatch repair
N^3-meA	=	N^3-methyladenine	MGMT	=	O^6-methylguanine - DNA methyl transferase
N^7-meG	=	N^7-methylguanine			
BER	=	base excision repair	DSB	=	double strand breaks
PARP	=	poly (ADP-ribose) polymerase			

amounts of MGMT are found in both normal and tumor cells. MGMT repairs the O^6-meG lesion by transferring the methyl group from the adduct to its own cysteine residue. Methylated MGMT is then degraded. Thus, MGMT is considered a 'suicide' repair protein, and new MGMT must be synthesized in order to continue DNA repair. Several

mechanisms exist to deplete MGMT in tumor cells in an attempt to increase sensitivity to TMZ (Fig. 2).

Strategies for overcoming resistance

Alternative schedules

As noted above, TMZ activity is schedule dependent, and the usual administration schedule is daily for 5 days, every 28 days. Because MGMT is depleted and not 're-usable' when it repairs the O^6-meG lesion induced by TMZ, it is suggested that changing the schedule of administration of TMZ can deplete more MGMT, thus enhancing the cytotoxic effect. Tolcher et al. [26], investigated different schedules of TMZ and the resultant effects on MGMT, as measured in peripheral blood mononuclear cells (PBMC). Patients received TMZ once daily for 7 days, every 14 days; or once daily for 21 days, every 28 days. It should be noted that MGMT activity in PBMC may not accurately reflect activity in tumor tissue. Nonetheless, with the schedules employed in this study, MGMT activity was significantly decreased at 7 days, and in the daily for 7 days group, activity remained decreased for seven more days.

Balmaceda et al. [27], evaluated an alternative schedule of TMZ in 120 patients with recurrent GBM, AA or anaplastic oligodendroglioma. TMZ was administered twice daily—as a 'bolus' dose of 200 mg/m^2 followed by 100 mg/m^2 every 12 h for nine doses (ten doses total). The trial did not meet the primary endpoint of a 50% improvement in progression-free survival (PFS). But the results (e.g., 6-month PFS of 35% in GBM) do compare favorably with historical data, and the twice a day dose was associated with acceptable toxicity.

These and other alternative schedules of TMZ continue to be investigated, based on the premise that more dose intense regimens (i.e., more drug delivered per unit of time) will be more efficient in depleting MGMT [28].

In addition to more effective inhibition of MGMT, altered schedules of TMZ might also inhibit angiogenesis. Metronomic scheduling refers to the administration of frequent low doses of chemotherapy [29]. It is suggested that metronomic dosing of cytotoxic chemotherapy, including TMZ, can block angiogenesis by inhibition of endothelial cells (with an intact genome) as well as inhibit proliferating tumor cells. Preclinical studies have demonstrated that exposure to low concentrations of TMZ inhibit angiogenesis and enhance apoptosis [30, 31]. Table 1 lists several common and alternative TMZ dosing regimens.

In a recent study conducted by the Medical Research Council (MRC BR12), chemotherapy naïve patients with recurrent high-grade gliomas were randomized 2:1:1 to receive procarbazine, CCNU and vincristine (PCV), traditional TMZ dosing (200 mg/m^2 daily for 5 days every 28 days), or TMZ 100 mg/m^2 daily for 21 days [32]. Median survival was similar for PCV versus both TMZ arms (6.7 vs. 7.2 months). However, in this trial, survival on the TMZ 5-day arm was superior to the 21-day arm (8.5 vs. 6.6 months, hazard ratio of 1.32, $P = 0.06$). From this randomized study the traditional 5-day schedule was superior to a dose intense regimen. RTOG 0525/EORTC intergroup phase III study is also addressing the optimal treatment schedule and results should be available in late 2009 or 2010. This study for newly diagnosed GBM has closed for enrollment and has over 1,100 patients stratified on MGMT and RPA status. They are initially treated with the standard EORTC-NCIC regimen of concurrent TMZ and RT followed by randomization of TMZ dosing in the traditional 5- or 21-day schedule as in the MRC BR12 study for six cycles.

Pharmacologic inhibitors of MGMT

As described above, MGMT repairs the O^6-meG lesion, leading to resistance to TMZ. O^6-benzylguanine (O^6-BG) serves as a substrate of MGMT [33], transferring its benzyl

Table 1 Temozolomide dosing regimens (all doses are oral)

Newly diagnosed	
With concurrent radiation	75 mg/m^2 per day × 42 days
Maintenance following radiation	Cycle 1: 150 mg/m^2 per day × 5 days
	Cycles 2 through 6: 200 mg/m^2 per day × 5 days, every 28 days
Recurrent disease	
No prior chemotherapy	200 mg/m^2 per day × 5 days, every 28 days
Prior chemotherapy	150 mg/m^2 per day × 5 days, every 28 days
Alternative schedules	50–175 mg/m^2 per day × 7 days, every 14 days
	75 mg/m^2 per day × 21 days, every 28 days
	200 mg/m^2 × 1 dose, followed by 90–100 mg/m^2 every 12 h × 9 doses, every 28 days

group to the cysteine residue of MGMT, thus using up MGMT. O^6-BG inhibits MGMT activity in bone marrow cells as well as tumor cells, and the main limitation to the clinical use of O^6-BG has been increased myelosuppression over that seen with an alkylator alone [34]. Phase I studies have investigated TMZ/O^6-BG combinations that would inactivate MGMT and maintain tolerability [35, 36]. Minimal efficacy data for these combinations are available.

Lomeguatrib depletes MGMT similarly to O^6-BG. Preclinical data suggest that lomeguatrib is associated with less myelosuppression than O^6-BG, and that lomeguatrib does not improve sensitivity to TMZ in cells with deficient mismatch repair (MMR) capacity [33, 34]. This illustrates the importance of the MMR system in TMZ cytotoxicity and resistance (Fig. 2).

Recent studies have also evaluated the clinical use of lomeguatrib, which can be administered orally. Dose reduction of concurrently administered TMZ due to MGMT inhibition in bone marrow is required, but not to the degree required with O^6-BG. TMZ has been given at two-thirds of the usual dose, when co-administered with lomeguatrib. MGMT activity in PBMC (thought to reflect the activity in bone marrow progenitor cells) and in various tumor cells was reduced by more than 95% with an oral dose of 40 mg lomeguatrib [37]. Disappointing results have been reported with the TMZ/lomeguatrib combination in advanced colon cancer and metastatic melanoma [38, 39]. However, the association between MGMT levels and clinical outcomes in CNS tumors is better established than that for colon cancer or melanoma. Thus, studies to determine an effective schedule of administration with the combination of lomeguatrib plus TMZ in CNS tumors are eagerly awaited.

Temozolomide and mismatch repair

The MMR system recognizes and repairs replication errors in newly synthesized DNA, including base–base mismatches [22]. As noted above, cells with deficient MMR are 'tolerant' to TMZ methylation (i.e., the cells are resistant to the effect of TMZ methylation). The 'futile' repair model describes the current thinking regarding the role of MMR in TMZ-induced cell death (Fig. 2). According to this model, the MMR system recognizes O^6-meG:T incorrect base pairs, as well as O^6-meG:C 'correct' base pairs but containing the abnormal methylguanine. But the cause of the error, methylated guanine, is in the template DNA strand, not the new strand. And MMR targets the new strand. Thus, the new strand with the error is degraded but the mismatch continues to occur when the 'wrong' base is again inserted to pair with O^6-meG. This sets up a repeating 'futile repair' cycle, eventually leading to DNA double strand breaks and cell death via apoptosis or autophagy. Cells deficient in MMR do not undergo apoptosis, and they survive with the DNA mutation. While the MMR system plays an important role in TMZ cytotoxicity and in resistance to TMZ, unlike the case with MGMT, there are no therapeutic strategies for directly overcoming or correcting MMR deficiencies.

Temozolomide and base excision repair

The base excision repair (BER) system, however, may offer a therapeutic strategy for increasing the sensitivity of tumor cells to TMZ. The BER system repairs the N^3-methyladenine (N^3-meA) adduct that accounts for only about 9% of TMZ-induced DNA adducts (Fig. 2). The N^3-meA lesion is highly lethal if it is not repaired by the BER system. Repair by the BER system is independent of MMR. Poly (ADP-ribose) polymerase (PARP) is an important component of the BER process [40]. PARP forms polymers on proteins that change the structure and function of the proteins, including proteins involved in the process of BER [22]. Most tumor cells do have an intact BER system, so generally, TMZ-induced N^3-meA lesions are easily repaired and do not result in cytotoxicity. However, if the BER system is inhibited, TMZ cytotoxicity mediated by N^3-meA lesions may be enhanced.

Several compounds that inhibit PARP, and thus the BER system, are currently being investigated in gliomas and other tumors [40, 41]. PARP inhibition has been demonstrated to restore sensitivity to TMZ in cells that have deficient MMR, independent of MGMT status [22, 40]. Exploitation of the N^3-meA lesion and inhibition of PARP may thus be particularly important in MMR deficient cells. PARP inhibitors in development include CEP-8983 and ABT-888 [40, 41]. Perhaps two mechanisms of TMZ-induced cytotoxicity can be enhanced by using a three-drug combination of TMZ, MGMT inhibitor and PARP inhibitor, in cells with increased MGMT activity.

In summary, MGMT remains a primary mechanism of resistance of CNS tumors to TMZ. The most effective way to inhibit MGMT and improve clinical outcomes is still to be determined. Active exploration of different schedules of TMZ with or without an MGMT inhibitor and combinations of TMZ plus MGMT inhibitor plus PARP inhibitor may lead to regimens that realize the full potential of TMZ activity against these tumors.

Adverse events

Myelosuppression is the dose limiting toxicity of TMZ. From the EORTC-NCIC trial, the rates of lymphopenia (grades 3/4) are 55%, thrombocytopenia (grades 3/4) 4–19%, neutropenia (grades 3/4) 8–14%, and leukopenia (grades 3/4) 11%.

Pneumocystis jiroveci (carinii) pneumonia (PCP) has been associated with TMZ use [8]. The risk of PCP is increased in patients receiving concomitant RT and with prolonged dosing. TMZ causes lymphopenia; low CD4 cell counts as well as concurrent use of corticosteroids contribute to immunosuppression, increasing the risk of PCP [42]. All patients receiving TMZ should receive prophylaxis for PCP during concurrent RT [8].

Other common adverse events associated with TMZ include nausea and vomiting, fatigue, alopecia, and constipation [1]. Nausea and vomiting occur in about 30% of patients, and are generally considered to be of mild to moderate severity. Administration of TMZ at bedtime may help to minimize these symptoms.

Aplastic anemia appears to be a growing concern with the use of TMZ. It is currently listed as a warning in the TMZ label. The exact incidence of aplastic anemia is unknown. According to Schering–Plough's safety database (personal communication, Schering–Plough Global Pharmacovigilance), as of December 13, 2007, 25 cases of aplastic anemia have been reported. Schering–Plough estimates the frequency to be 10.22 per 100,000 patients exposed to TMZ. From August 11, 1999 to November 3, 2006, the FDA received 18 (domestic 14, foreign 4) reports of aplastic anemia [43]. The mean age of patients in these case reports was 48 years, with ten of the 18 patients receiving TMZ for GBM. The median time to onset of aplastic anemia from the start of TMZ therapy was 36 days (range 5–578) and nine cases occurred in treatment-naïve patients receiving TMZ according to the labeled dose [43]. Other drugs such as chemotherapeutic agents, anticonvulsants, and cotrimoxazole may also contribute to bone marrow suppression or blood dyscrasias. According to the FDA, six of the 18 cases indicated prior or concurrent exposure to these medications. Several cases of aplastic anemia, some of which may be included in the Schering–Plough or FDA databases, have also been published [44–48].

Severe and potentially irreversible thrombocytopenia has also been noted [49]. In addition, a few cases of myelodysplastic syndrome or AML have been reported [50, 51]. Thus, the risk of severe treatment-related hematologic toxicity is small but significant, and should be disclosed to patients.

Pseudo-progression

The improved clinical outcome with the Stupp regimen has come with reports of early radiation-induced imaging changes with increased enhancement, termed pseudoprogression [52–55]. The etiology of this radiation effect is presumed to be increased vascular permeability secondary to treatment-induced inflammation and not the full development of radiation-induced necrosis [55]. The historical incidence of necrosis in patients treated with radiation alone is <5% [56]. Numerous cohort-based studies using the Stupp regimen demonstrate increased enhancements with an incidence of up to 50% [52–55]. However, a caveat is that these studies lack direct comparison with RT alone and although the incidence is likely increased it is unknown [57]. This effect following chemoradiation occurs earlier than that seen historically for radiation necrosis: <12 weeks following combined radiation/TMZ therapy, versus 6 months to 1 year or longer following radiation alone (Fig. 3) [52–55]. The findings of an enhancing mass with associated edema are indistinguishable from recurrent tumor by current imaging standards [52]. Brandes et al. [54], postulate that pseudo-progression may have caused an overestimation of disease progression in the EORTC-NCIC study in which PFS (6.9 months with the Stupp regimen and 5 months with radiation alone) was rather modest relative to overall survival (27 vs. 10% at 2 years) [1].

The increase in radiation necrosis has made clinical management difficult. The usual method of evaluating brain tumor response to treatment, the Macdonald criteria, utilizes imaging, along with neurologic functional status and corticosteroid use [58]. Neuro-oncologists are also aware that seizures can bring about changes in enhancement [59]. Clinically, patients with pseudo-progression seem to have less neurologic deterioration than those with true progression, but this is not always the case [52–55]. Patients with pseudo-progression are also more likely to have *MGMT* promoter methylation and increased survival [54], likely reflecting increased effectiveness of treatment. Surgical resection is the only definite method of distinguishing pseudo-progression from true progression, but this is often impractical. Corticosteroids are usually administered or continued, and they provide symptomatic benefit, although the long-term benefit is largely unknown. Recent reports suggest that bevacizumab can improve radiologic signs of pseudo-progression, presumably by normalizing vascular permeability, although the clinical impact is unknown [60]. A revision of the Macdonald criteria to incorporate pseudo-progression is currently in progress.

In general, recognition of the high incidence and the clinical characteristics of pseudo-progression should guide clinicians to continue TMZ therapy when possible during adjuvant treatment. Clinical trials evaluating imaging modalities such as magnetic resonance (MR) spectroscopy, MR perfusion, and positron emission tomography (PET) scans are being conducted.

Length of adjuvant TMZ therapy

In the Stupp regimen, TMZ is administered for six cycles as adjuvant or maintenance therapy. Many neuro-oncologists,

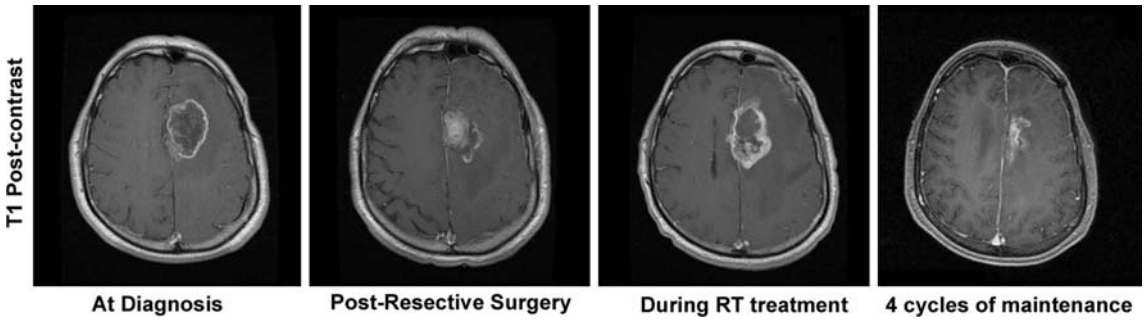

Fig. 3 Clinical course of pseudo-progression with MRI post-contrast study. T1 weighted MRI axial view with Gadolinium contrast

however, administer TMZ for longer periods—some even advocate continuing treatment until disease relapse [54, 61]. Longer treatment periods are chosen because residual disease is often present or suspected, and because of the relatively short time to relapse in the chemoradiation arm of the EORTC-NCIC trial (median 7 months from diagnosis). For the most part TMZ is tolerable, with up to 40 cycles of treatment given with only limited cumulative toxicity [61]. However, the potential for severe hematologic toxicity must be considered with prolonged use. Thus, the optimal length of adjuvant therapy remains controversial. With limited data available in support of prolonged therapy, decisions about the duration of treatment can be difficult for both patients and physicians. It is noteworthy that six cycles was chosen based on adjuvant therapy for breast and colon cancers [62], and in no other solid tumor is adjuvant or maintenance cytotoxic chemotherapy administered for longer than 1 year or as a single drug. Therefore, we recommend six cycles of adjuvant therapy. In select cases longer treatment may be appropriate, based on imaging and clinical factors.

Conclusion

Temozolomide represents a significant advance in neuro-oncology and the encouraging results of the EORTC-NCIC phase III study have stimulated further interest and research in the field. Large phase III trials evaluating novel therapies, including angiogenesis inhibitors, in combination with the Stupp regimen are underway [62]. Still to be resolved are questions about the diagnosis and management of pseudo-progression, the length of maintenance therapy, the value of routine MGMT testing for TMZ sensitivity, and the nature and incidence of irreversible bone marrow suppression. Nonetheless, TMZ has contributed much to an improved outlook for patients with gliomas.

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