Optimal Role of Temozolomide in the Treatment of Malignant Gliomas

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Temozolomide (TMZ) is an alkylating agent that was approved for anaplastic astrocytoma and glioblastoma. Its role in the treatment of recurrent disease has been confirmed, and more importantly, alternative treatment schedules and combination regimens have been developed. A recent phase III trial has demonstrated a survival advantage for concomitant TMZ administration with radiotherapy in patients with newly diagnosed glioblastoma. Molecular studies suggest a strong predictive role of the DNA repair enzyme O6-methyl-guanine-DNA-methyl-transferase (MGMT) and outcome of TMZ-based chemotherapy. This review summarizes the current knowledge, highlights approved and nonapproved indications, and describes molecular studies that may allow us to identify the patients most likely to benefit from this treatment.

Introduction

Temozolomide (TMZ) has emerged as an active agent against malignant glioma over the past decade [1]. TMZ was approved 5 years ago in both the United States and Europe, based on only three phase II trials, for the treatment of recurrent anaplastic astrocytoma, and it was also approved in Europe for the treatment of recurrent glioblastoma [2–4]. The use of temozolomide concomitant with radiotherapy in glioblastoma received Food and Drug Administration approval in March 2005. Antitumor activity of temozolomide against malignant glioma has been confirmed in clinical practice [5] and recently in a phase III randomized trial [6••]. In addition to the standard dosing of TMZ (150 to 200 mg/m^2/d for 5 days every 4 weeks), novel, continuous low-dose and dose-dense regimens have been developed [7].

With growing experience and application to a large range of gliomas many new questions are arising. What is the best dosing regimen? What is the optimal duration of therapy? Can the results obtained in glioblastoma be extrapolated to all types of glioma? This review summarizes the current state of knowledge illustrated by selected recent references. We emphasize practical aspects and the available evidence, highlight some novel developments, and discuss directions of future research.

Pharmacology and Mechanisms of Action

Temozolomide is an oral alkylating agent that is rapidly and completely absorbed and spontaneously converts into the active metabolite MTIC. TMZ has linear pharmacokinetics with maximum plasma concentrations 30 to 90 minutes after oral intake. Although recommended to be taken in a fasting state (at least 1 hour before and after food intake), food will only lead to a 10% reduced area under the concentration curve (AUC) and a delayed peak concentration [8]. TMZ has an excellent penetration into all body tissues. Pharmacokinetics of TMZ in the cerebrospinal fluid (CSF) have been performed in a recently reported study [9•]. The AUC in the CSF as a surrogate for central nervous system penetration corresponded to approximately 20% of the AUC in the plasma [9•]. Similar to nitrosoureas, TMZ acts as a DNA alkylating agent. Methylation of the O-6 position of uanine yields one of the most biologically important, although not most frequent, adducts. If left unrepaired, this lesion triggers cytotoxic response and apoptosis. Cells have the capacity of restoring unmethylated guanine through the DNA excision repair enzyme O6-methyl-guanine-DNA-methyl-transferase (MGMT), also known as O6-alkyl-guanine-alkyltransferase (AGAT), which removes the methyl group from the O-6 position. During this process, the enzyme is consumed and requires subsequent resynthesis. High endogenous MGMT activity in cancer cells blunts the treatment effect of alkylating agent chemotherapies, creating a resistant phenotype [10••,11] and may be an important determinant of treatment failure [12–15]. A significant number of brain tumors express low concentrations of this enzyme [12,14].

Continuous administration of TMZ will lead to gradual consumption of all MGMT, thus exhausting the cell's repair mechanism possibly by overcoming this inherent resistance [16••]. Feasibility and activity of protracted TMZ administration schedules are discussed in the following text (Table 1).

Table 1. Temozolomide administration schedules

Schedule	Dose, mg/m^2	Dose intensity, $mg/m^2/wk$	Study
Daily for 5 d, repeat every 28 d	(150)–200	250	Package insert
Daily for 7 d, repeat every 14 d	150	525	Tolcher *et al.* [16••], Wick *et al.* [27]
Daily for 21 d, repeat every 28 d	100	525	Tolcher *et al.* [16••]
Daily for 42 d, repeat every 70 d	75	315	Brock *et al.* [7]
Daily for 3 d, repeat every 14 d	300	450	Vera *et al.* [17]
Twice daily for 5 d, repeat every 28 d	200 (bolus), then 90 for 9 doses	250	Spiro *et al.* [18]

Temozolomide Schedules

In vitro and phase I studies have established the initial standard dosing of TMZ at 150 to 200 $mg/m^2/d$ for 5 days every 4 weeks. This was shown superior to an equivalent total dose administered on a single day. Novel continuous administration schedules have since been developed, allowing for more intensive and dose-dense regimens. Using these low-dose daily schedules, the dose-intensities could be increased to over twofold (Table 2) [7,16••17,18]. To date, there are no conclusive data available demonstrating that the denser, low-dose continuous schedules offer clinically relevant increased antitumor activity, despite considerable theoretical appeal [19].

One additional particularity of the continuous administration schedules is induction of profound lymphocytopenia, with CD4 counts similar to AIDS patients [20]. The common use of corticosteroids in brain tumor patients further adds to an immunodeficient state, predisposing to opportunistic infections [21]. Increased incidence of opportunistic infections, in particular *Pneumocystis carinii* pneumonia and Kaposi sarcoma, have been reported [22•,23••]. Monitoring of CD4 counts and antibiotic prophylaxis with trimethroprim/sulfametoxazole or pentamidine inhalations has been proposed [22•,23••,24].

Trial Methodology

The gold standard to prove or disprove efficacy of a chemotherapy agent remains a large phase III trial demonstrating a clear survival benefit. As a surrogate, shrinkage of the tumor size by 50% or more (area of two perpendicular diameters on computed tomography or magnetic resonance imaging) has been shown to be a valuable endpoint (World Health Organization criteria); in the brain, the dose in corticosteroids also needs to be stable or decreasing (Macdonald criteria) [25]. In the brain, responses may be delayed and are occasionally only seen many months after treatment starts. Objective response rates in recurrent malignant glioblastoma are commonly below 10%, and are between 10% and 20% for anaplastic astrocytoma. As stabilization of a glioblastoma for a prolonged period of time is of benefit for the patient, and as progression is often easier to identify than measuring percentage of tumor shrinkage, progression-free survival (PFS) at 6 months has been suggested as a surrogate endpoint. Based on the pooled results of eight consecutive and negative phase II trials in patients with grade III and grade IV astrocytoma, this new endpoint was proposed [26]. The authors suggested considering a rate of PFS at 6 months of 15% for glioblastoma and of 31% for anaplastic astrocytoma as a benchmark for comparison in phase II trials [26]. Most current trials of new chemotherapy agents for recurrent glioma refer to this endpoint; however, validation in a prospective randomized trial or an independent dataset is still lacking.

In the clinical practice of oncology, the aim is commonly palliation of symptoms and improvement or maintenance of quality of life. Thus, in rapidly growing and devastating diseases, even disease stabilization may be of therapeutic value and requires clinical judgment beyond the crude numbers of response rates in clinical trials. However, in some reports of clinical trials, disease stabilization has repeatedly been included in the overall response rate. This is a deceiving practice when the duration of stabilization is not taken into account. In many of those reports, the duration of this stabilization is no longer than the time to the first response assessment after only 2 months of therapy.

Temozolomide in Recurrent Glioma

The efficacy of TMZ was initially demonstrated in patients with recurrent disease. Three pivotal phase II studies with identical entry criteria were conducted and, for the first time, separate studies were conducted for patients with glioblastoma and with anaplastic astrocytoma. Despite disappointingly low objective response rates in glioblastoma of 5% and 7%, respectively, and with a response rate of 35% in the anaplastic astrocytoma trial, these studies suggested an increase in PFS at 6 months compared with a historical database. Subgroup analysis suggested that patients who had not received prior adjuvant chemotherapy benefited from this treatment. In one recent report, TMZ (150 $mg/m^2/d$) was administered on an intensive schedule of 7 days on and 7 days off to 21 eligible patients with recurrent or progressive

Table 2. Temozolomide in oligodendrogliomas

Histology	Cases, *n*	Response rate	1-y PFS	Remarks	Study
Astrocytoma (2), AOA (14), AOD (23)	39	53%	40%	First-line therapy	van den Bent *et al.* [28•]
AOA (11), AOD (17), other (4)	32	31%	11%	Failed previous PCV after initial response in 31%	van den Bent *et al.* [31]
AOA (9), AOD (39)	48	44%	25%	Failed previous PCV after initial response in 44%	Chinot *et al.* [30]

AOA—anaplastic mixed oligoastrocytoma; AOD—anaplastic oligodendroglioma; PCV—procarbazine, lomustine (CCNU), vincristine; PFS—progression-free survival.

glioblastoma. An objective response was observed in two patients (10%), and at 6 months 43% of patients were progression free and 1-year survival was 81% [27]. Although these results appear promising, confirmation in a larger, well-controlled trial is required. The authors excluded seven patients (25%) from the analysis, thus introducing a potential important bias.

Oligodendroglioma, and to a lesser extent, oligoastrocytoma, are considered chemoresponsive diseases. Several phase II trials investigated the role of TMZ in patients with recurrent anaplastic oligodendroglioma and oligoastrocytoma after prior radiotherapy [28•,29,30]. The European Organization for Research and Treatment of Cancer (EORTC) conducted two phase II trials evaluating single-agent, standard-schedule TMZ as first- and second-line therapy in patients with recurrent or progressive anaplastic oligodendroglioma andoligoastrocytoma [28•,31]. High response rates of 53% (26% complete responses) and 25% were observed as first- and second-line chemotherapy, respectively. Most patients that responded to second-line therapy had also responded to first-line PCV chemotherapy (procarbazine, lomustine [CCNU], vincristine), but some patients that do not respond to PCV may still respond to TMZ [31]. Other studies showed a large variability of response rates in second-line studies after prior PCV chemotherapy (between 25% and 44%). This probably reflects the importance of patient selection, in particular concerning cases of "true" oligodendroglioma. Regardless of the sequence of treatment (*ie*, TMZ first or PCV first and the other agent as second-line therapy), the response rate in first-line therapy is about 55% to 70%, and in second-line therapy is 17% to 26% [32]. This implies that even chemotherapy-sensitive oligodendroglioma has a limited sensitivity to second-line treatment, and that a further improvement of medical treatment requires novel agents.

A correlation of chemotherapy response and loss of heterozygosity (LOH) on chromosomes 1p/19q has previously been suggested, with close to 100% of patients responding to PCV chemotherapy [33]. First results of similar studies on TMZ chemotherapy confirm the importance of 1p/19q loss for response to this drug [32].

Chemotherapy As First-line Treatment of Oligodendroglioma and Gliomatosis

Currently, a tendency exists to give TMZ as first-line chemotherapy even before radiotherapy to patients with pure oligodendroglioma and LOH 1p/19q. This practice shift is not supported by true evidence of superiority, and many of these tumors may respond very well to radiotherapy [34]. For smaller tumors in particular, primary radiotherapy can be administered without significant risk for delayed toxicity and may be the simpler therapeutic choice. Primary chemotherapy requires close follow-up, excellent patient compliance, and repeat magnetic resonance imaging. However, for larger tumors involving several lobes (gliomatosis cerebri) and requiring large radiotherapy fields, primary TMZ chemotherapy may be preferred [35]. Sanson *et al*. [36•] summarized the French experience of 46 patients treated with TMZ and 17 patients receiving PCV for gliomatosis cerebri. A clinical benefit was suggested for both regimens, with a PFS and overall survival of 16 and 29 months, respectively. Oligodendroglial gliomatosis had a significantly better prognosis.

Temozolomide for Patients with Newly Diagnosed Glioblastoma

Based on the definite, albeit modest, activity in the treatment of recurrent glioma, we investigated the administration of TMZ in patients with newly diagnosed glioblastoma. The administration of TMZ early in the disease course to a patient with a good performance status will allow for a sufficiently long drug exposure, and a less-resistant tumor phenotype. In vitro data also suggested additive or supra-additive activity when TMZ was administered concomitantly with radiotherapy. The intrinsic antitumor activity of TMZ may also affect infiltrating tumor cells outside the radiation field (spatial cooperation). In a phase II trial, the administration of low-dose TMZ daily (75 mg/m^2/d for 7 days a week) with concomitant radiotherapy for up to 7 weeks, followed by six cycles of adjuvant TMZ (5 consecutive days every 28 days) was shown to be feasible, was well tolerated overall, and initial results were promising [22•]. The EORTC and the National

Cancer Institute of Canada (NCIC) Clinical Trials Group conducted a large phase III trial with a total of 573 patients comparing this new combined-modality regimen with standard radiotherapy alone [6••]. The primary trial endpoint was survival. This trial unequivocally demonstrated that the combination of TMZ and radiotherapy followed by up to six cycles of adjuvant TMZ improves survival. The risk of death was reduced by 37%, translating in a prolongation of the median survival from 12.1 to 14.6 months, and survival at 2 years increased from 10% to 26%. Similarly, PFS was also prolonged in the patients treated with TMZ and radiotherapy. The applicability of these results to everyday practice is illustrated by the fact that patients were randomized from 85 institutions in 15 countries. Subgroup analyses suggested that patients of all age groups (up to age 70 years allowed in the trial) and independent of prior tumor resection benefited from this treatment. As in other fields of oncology, patients with a considerably impaired performance status improved least from the addition of chemotherapy. In this trial, only 40% of the patients received all planned six cycles of adjuvant TMZ after the end of TMZ and radiotherapy. The main reason for early discontinuation was disease progression.

This trial was not designed to distinguish whether it is the concomitant administration of chemotherapy and radiation therapy, or the adjuvant TMZ administration, contributing most to the improved outcome. Similarly, this trial cannot answer whether prolonged administration of TMZ beyond the six planned cycles would further improve the outcome. Although administration of TMZ for up to 2 years has been shown safe and without cumulative toxicity, we stop treatment after six adjuvant cycles. Tumors recurring after a free interval of more than 3 to 6 months could be considered for retreatment with TMZ, but clinical data are scarce. Occasional minor responses have been observed, and tumor growth stabilization often can be achieved for several months. Novel strategies are clearly needed.

Temozolomide As First-line Therapy Before or Instead of Radiotherapy

High response rates with first-line TMZ chemotherapy immediately after surgery or biopsy have been reported. Gilbert *et al*. [37] reported on 36 glioblastoma patients receiving standard-dose TMZ for up to four cycles. A complete response was observed in four patients and a partial response in 11 patients, for an overall response rate of 42% with a median PFS survival of 4 months and overall survival of 13 months. Similar results were previously reported by Friedman *et al*. [38]. Of 33 glioblastoma patients, three had a complete response, 14 had a partial response, and 12 progressed, accounting for a 52% response rate and a 36% rate of progression. Overall survival or time to progression was not reported [38]. With neoadjuvant combination chemotherapy of BCNU (carmustine), a response rate of 43% (95% CI, 27% to 58%) and PFS and overall survival of 7 months and 13 months, respectively, were observed in a French trial [39•]. The North American Brain Tumor Consortium (NABTC) reported disappointing response rates and high toxicity for a similar combination of BCNU followed by a single dose of TMZ (550 mg/m^2) in patients with anaplastic astrocytoma and oligodendroglioma [40]. The response rate was 29% (94% CI, 16% to 46%). We administered four cycles of TMZ on a continuous dose-dense schedule (100 mg/m^2 for 3 weeks out of 4) to 20 patients with newly diagnosed glioblastoma before concomitant TMZ and radiotherapy. In a preliminary analysis of our experience we only observed rare objective responses; however, overall survival is comparable with our experience with the standard sequence of TMZ and radiotherapy followed by TMZ (Stupp, Unpublished data].

One phase II trial evaluated the administration of TMZ in 32 elderly patients with a median age of 75 years [41]. The response rate was 31% (95% CI, 14% to 48%) and the median survival was 6.2 months, comparable with the 5.2 to 5.6 months recently reported for radiotherapy alone [42]. A randomized trial by the Nordic Neuro-oncology group comparing radiotherapy with a standard dose of TMZ, is ongoing.

Evaluating new treatment regimens permits testing new agents with a maximum chance for success and allows researchers to evaluate the activity of the new agents or combinations in a homogeneous patient population. However, the common practice of administering TMZ after surgery for a variable time period and outside of clinical trials to compensate for undue delays in initiating radiotherapy is not justified. The early randomized trials have shown without doubt that radiotherapy is superior compared with chemotherapeutic treatment with BCNU only [43]. Radiotherapy should start within 4 to 6 weeks of surgery.

Temozolomide in Combination with Other Cytotoxic Agents

Due to its favorable safety profile, TMZ is an attractive agent for combination chemotherapy regimens with other active agents. In combination with other alkylating agents (*eg*, BCNU), increased and schedule-dependent toxicity is to be expected due to fact that repair of the DNA damage induced by both agents depends on MGMT.

Combination chemotherapy of BCNU (150 mg/m^2) and TMZ (110 mg/m^2/d for 5 days, repeat every 42 days) was shown to be feasible, although 15% of patients experienced severe grade 3/4 hematotoxicity [39•]. The NABTC reported prohibitive toxicity for three regimens: 1) BCNU (150 mg/m^2) followed by a single dose of TMZ (550 mg/m^2 on day 1, repeated every 42 days); 2) BCNU (200 mg/m^2 on day 1) and TMZ (150 mg/m^2 on days 1 to 5, repeat every 42 days); and 3) TMZ (150 mg/m^2 on days

1 to 5) and BCNU (150 mg/m^2 on day 1, repeat every 56 days) [40]. Hematologic toxicity was observed in approximately half of the patients, and pulmonary toxicity was observed in particular in the arm receiving BCNU before TMZ. The lung toxicity that was observed (a known complication of BCNU therapy) is probably due to the higher dose of BCNU, as this observation was not made in a similar regimen with lower doses as reported by Barrie *et al.* [39•]. A phase I trial determined a fixed dose of BCNU (150 mg/m^2 on day 1) followed by TMZ (80 mg/m^2 continuously for 28 days), as recommended doses for phase II evaluation. However, repeated dosing may be difficult due to cumulative myelosuppression [44]. TMZ (130 mg/m^2 bolus followed 70 mg/m^2 twice daily for 5 days) and cisplatin (75 mg/m^2) were evaluated in 50 chemo-naive patients with recurrent glioblastoma [45]. Grade 3/4 myelosupession was observed in 15% of patients. A response rate of 20% (95% CI, 8% to 33%) and a rate of PFS at 6 months of 34% (95% CI, 23% to 50%) was reported. Overall survival was 11.2 months (95% CI, 9.7% to 14.0%). These results compare favorably with the pivotal trials of TMZ as a single agent, with response rates of only 5% to 7%, a PFS at 6 months of 20%, and overall survival of 5.4 to 7.3 months [46]. The advantage of combining cisplatin with temozolomide is the non-overlapping toxicity profiles, allowing treatment with the full dose of both agents. However, the addition of cisplatin negates the substantial advantage of safe and convenient outpatient administration of TMZ. Therefore, carboplatin may be an alternative worth exploring, but confirmation of superiority of the combination in a randomized trial is necessary.

Since the initial report of activity of irinotecan (CPT11) in recurrent malignant glioma [47], this agent has been investigated in various combinations, including BCNU [48] and TMZ [49]. As irinotecan is mainly metabolized in the liver dependent on the P450 system, it can only be reliably administered to patients not requiring concomitant therapy with hepatic enzyme-inducing antieptileptic drugs (EIAED).

The Italian Neuro-Oncology Cooperative Group treated patients on EIAEDs who had failed prior radiotherapy and TMZ with a combination of an escalated dose of CPT11 (175 to 200 mg/m^2 weekly for 4 weeks) and BCNU (100 mg/m^2 on day 1, repeat cycles every 6 weeks) as salvage chemotherapy [48]. They report a response rate of 21% (95% CI, 9% to 34%) and a PFS rate at 6 months of 30% (95% CI, 19% to 50%). A pediatric phase I trial demonstrated the absence of relevant drug interactions with TMZ (100 mg/m^2) and CPT11 (10 mg/d intravenously by 10 doses on days 1 to 5 and days 8 to 12). Dose-limiting toxicities were infection, diarrhea, and myelosuppression [50]. In a preliminary report, Jones *et al.* [51] summarized their experience of a phase I trial in patients with solid tumors. They explored three different dosing regimens: 1) TMZ on days 1 to 14 and CPT11 on day 1, repeat every 28 days; 2) TMZ on days 1 to 7 and 15 to 21 and CPT11 on days 8, 15, 22, repeat every 42 days; or 3) TMZ days 1 to 7 and 15 to 21 and CPT11 on days 1 and 15, repeat every 28 days. Schedule 2 was abandoned due to a high frequency of grade III diarrhea. Dose-limiting toxicity of diarrhea and myelosuppression were also encountered in schedule 1 at dosagees of 125 mg/m^2 of TMZ and 250 mg/m^2 of CPT11. We are conducting a phase I trial in patients with recurrent glioma who are not receiving EIAEDs. We were able to escalate both drugs to single-agent maximum tolerated dose (MTD) and are currently treating patients with TMZ (100 mg/m^2 daily for 14 days) and CPT11 (350 mg/m^2 on day 8) every 3 weeks [49].

Temozolomide has also been or is undergoing evaluation in combination with noncytotoxic agents like the metalloproteinase inhibitors marimastat and prinomastat [52,53], thalidomide [54], cis-retinoic acid [55], and farnesyl-transferease inhibitors [56]. Despite a good theoretical rationale for all regimens, the available data from these phase II trials do not allow for any firm conclusions in regard to increased activity. In particular, the absence of any comparator in these studies is a major shortcoming, and the presumably improved outcome could just be a result of patient selection. A randomized phase II design may be a practical approach for future phase II evaluations.

Low-grade Glioma

Low-grade glioma may respond to chemotherapy. Biemond-ter Stege *et al.* [35] reported on their experience of treating 16 patients with low-grade oligodendroglioma and mixed oligoastrocytoma with primary PCV chemotherapy. Activity, in particular minor responses and a long time to progression (median not reached at > 24 months), was observed in 13 patients. Response rates of over 40% to 60% to TMZ chemotherapy have been reported in two reports of patients treated for progressive low-grade glioma (Table 3) [57,58]. However, inclusion in this trial was based on initial histology, and the presence of contrast enhancement in 60% to 70% of the patients and the confirmed transformation into anaplastic glioma in over 50% of the operated patients clearly indicates that most patients had a higher grade tumor and that the observed responses are in concordance with earlier reports [59]. There are two reports of TMZ administration (standard schedule) to patients with previously untreated low-grade glioma [60•,61••]. Objective response rates are 10% and 17%, respectively, with a 14% to 48% rate of minor responses or clinical improvement. The median time to maximal response in both studies was 12 months. These results suggest that TMZ does have activity for lower-grade glioma. However, whether there is an advantage in treating these patients with upfront chemotherapy for 12 months or longer compared with initial radiation therapy is currently the subject of a randomized EORTC/NCIC trial [59]. With the possible exception of oligodendroglioma

Table 3. Temozolomide in low-grade gliomas

Histology	Cases, *n*	Response rate	1-y PFS	Remarks	Study
Astrocytoma (17), OA (2), OD (11)	30	10% (+48% MR)	76% (at 2 y)	Primary therapy	Brada *et al.* [60•]
OA (11), OD (49)	60	17% (+14% MR)	73%	Many delayed responses	Hoang-Xuan *et al.* [61••]
Astrocytoma (16), OA (5), OD (20), other (5)	46	61%	76%	Contrast +70%*	Quinn *et al.* [57]
Astrocytoma (29), OA (10), OD (4)	43	47%	39%	Contrast +60%*	Pace *et al.* [58]

*Contrast enhancement on computed tomography and/or magnetic resonance imaging.
MR—minor response; OA—mixed oligoastrocytoma; OD—oligodendroglioma; PFS—progression-free survival.

with 1p/19q, LOH patients with low-grade glioma should not receive TMZ as initial therapy outside a clinical trial. Similarly, despite the proven increased effectiveness for concomitant (and adjuvant) TMZ chemoradiotherapy, extending this approach to low-grade glioma is potentially harmful. In a disease with median survival rates of 5 to 7 years and a significant proportion of patients living 10 to 15 years or longer, late toxicity of any treatment is a concern. Here, the sequential and prudent use of the treatment modalities may be more optimal than a maximal use of all therapeutic arms up front.

Identifying Patients Likely to Benefit

A common feature of oligodendroglioma is the chromosomal loss on chromosomes 1p and 19q (LOH 1p/19q), which has been associated with higher response to PCV chemotherapy [33] and also to TMZ treatment [61••]. Out of a larger trial, tumor samples of 26 patients treated with TMZ were available for molecular analyses. LOH on 1p was detected in 12 patients. Six of the 12 patients had either a partial or minor response, the other patients remained stable. Of the 14 patients with intact 1p, no response was observed and one patient progressed [32]. Similarly, in EORTC study 26971 describing first-line chemotherapy with TMZ, 10 of 13 patients with 1p loss responded, in contrast to only one of six without 1p loss ($P = 0.04$) [28•,32]. These data also confirm the correlation of 1p loss and chemotherapy response for TMZ. However LOH on 1p may just be a surrogate marker for tumor responsiveness, independent of the actual treatment. Also, responses can be observed in a significant proportion of patients without 1p loss [62]. In a randomized Radiation Therapy Oncology Group (RTOG) trial, patients with anaplastic pure and mixed oligodendroglioma were randomized to receive or not receive four cycles of neoadjuvant PCV chemotherapy before radiotherapy [63]. Although overall survival was not different between the two groups, PFS was prolonged with neoadjuvant chemotherapy; the benefit could be attributed to a large extent to the subgroup with 1p/19q LOH.

The role of the DNA repair enzyme MGMT has been described previously in this paper. Epigenetic silencing by promoter methylation of the gene encoding the DNA repair enzyme MGMT has been associated with prolonged survival in patients treated with nitrosoureas [64•] or with TMZ [65]. However, will determination of the methylation status of the MGMT gene promoter help to identify the patients truly benefiting from TMZ chemotherapy, or is MGMT promoter methylation simply another prognostic factor independent of the treatments administered? In the randomized EORTC/NCIC trial, patients received either radiotherapy alone or TMZ and radiation therapy as initial treatment for GBM [6••,66••]. We determined the status of the MGMT gene promoter in tumor DNA derived from 206 patients using methylation-specific polymerase chain reaction. In 45% of the tumor samples, the MGMT gene promoter was methylated, thus the gene was epigenetically inactivated. Overall, patients with a silenced MGMT gene had a longer survival. When analyzing by treatment received and after adjustment for other prognostic factors, patients with a methylated MGMT receiving TMZ and radiation therapy survived the longest. In contrast, there was little difference in survival and no difference in PFS among patients with an unmethylated MGMT (and thus a functioning repair system) receiving TMZ and radiation therapy and patients receiving radiation therapy only, irrespective of their methylation status [66••]. In our analysis, the 2-year survival rate for patients with a methylated MGMT gene increased from 22% for patients receiving initial radiation therapy alone to 46% with combined chemoradiotherapy. In comparison, the 2-year survival for patients with unmethylated tumors was 2% and 13% only, with radiotherapy and with TMZ and radiotherapy, respectively. These data strongly suggest that MGMT is a predictive marker for benefit from TMZ chemotherapy. If confirmed, this may allow selecting the patients upfront. Only patients with a methylated MGMT promoter (and thus a silenced gene) should receive TMZ, whereas for other patients alternative and more appropriate strategies (*eg*, investigational agents, intensive dose-dense TMZ schedules, and agents depleting MGMT) could be considered.

Conclusions

Temozolomide has greatly extended our armamentarium in the treatment of malignant glioma. The early introduction of a cytotoxic agent with the capacity of crossing the blood-brain barrier and the administration of concomitant chemoradiotherapy have a clear impact on progression and survival of glioblastoma patients. Molecular studies have shown that specific tumor characteristics, such as a silenced MGMT gene, may allow us to tailor treatment for individual patients. New trials are planned to confirm these findings, but also to develop strategies allowing us to deplete the unmethylated tumors from MGMT, (*eg*, by using more intensive treatment schedules).

The current progress in the treatment of malignant glioma should fuel interest in further improving the outcome of patients with brain tumors. Optimizing current agents and treatment regimens and combination with new cytotoxic and targeted molecules holds promise. The close interaction and integration of laboratory findings and clinical research will allow us to identify new treatment targets and will be a prerequisite for individual treatment decisions in the future. The ongoing EORTC/NCIC randomized trial in low-grade glioma is a good example.

Optimal management of patients with brain tumors requires an integrated and multidisciplinary approach. Despite the recent progress and the successful introduction of temozolomide in clinical practice, further research and development of appropriate agents is still needed. Correlative laboratory studies will help us better understand the successes and failures.

Acknowledgments

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