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Review

Recent developments in the use of chemotherapy in brain tumours

Martin J. van den Bent[a,*], *Monika E. Hegi*[b], *Roger Stupp*[c]

[a]*Department of Neuro-Oncology, Daniel den Hoed Cancer Center/Erasmus University Hospital Rotterdam, P.O. Box 5201, 3008AE Rotterdam, The Netherlands*
[b]*Laboratory of Tumour Biology and Genetics, Department of Neurosurgery, University of Lausanne Hospitals, Lausanne, Switzerland*
[c]*Multidisciplinary Oncology Center, University of Lausanne Hospitals, Lausanne, Switzerland*

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ABSTRACT

Several recent studies have further clarified the role of chemotherapy in newly diagnosed anaplastic glioma. For newly diagnosed glioblastoma, combined daily radiotherapy with daily temozolomide followed by six cycles of adjuvant temozolomide improves overall survival. This benefit is especially observed in patients with a methylated promotor of the *MGMT* gene which encodes an alkyltransferase; this observation however, needs confirmation. Although oligodendroglial tumours are sensitive to chemotherapy, classical adjuvant nitrosourea-based chemotherapy does not improve overall survival in newly diagnosed anaplastic oligodendroglioma, even in the subset of 1p/19q loss tumours. It may increase progression-free survival however, and further studies must show if combined modality treatment with daily chemotherapy during radiotherapy increases survival. Trials exploring the role of chemotherapy in low-grade glioma are ongoing. No standard chemotherapy is currently available for highly anaplastic glioma failing first-line temozolomide-based therapy.

1. Introduction

Randomized trials on malignant glioma conducted from 1970 to the 1990s clearly established the role of radiotherapy in the treatment of anaplastic glioma, but all failed to demonstrate a significant increase in survival by the addition of chemotherapy (Table 1).[1,2] It took a large individual patient data based meta-analysis comprising over 3000 patients to show that adjuvant chemotherapy may improve the 2-year survival by only 5%, from 15% to 20%.[3] In recent years, the role of chemotherapy in glioma has been met with renewed interest mainly due to two developments: the recognition of the sensitivity to chemotherapy of 1p/19q loss oligodendrogliomas; and the availability of temozolomide, a novel alkylating agent with good penetration in the central nervous system. In the 1990s a series of phase II trials have shown activity of temozolomide (TMZ) against recurrent glioblastoma and anaplastic astrocytoma. After approval in 1999, TMZ has largely replaced nitrosoureas in the treatment of glioma, although a formal comparative trial was never conducted. Recently, a randomized trial has demonstrated a superior survival in newly diagnosed glioblastoma multiforme (GBM) patients, treated with TMZ and radiation.[4] Both PCV combination chemotherapy (procarbazine, CCNU and vincristine) and temozolomide were investigated as treatment for recurrent and newly diagnosed anaplastic oligodendrogliomas. This review

* *Corresponding author:* Tel.: +31 10 4391415; fax: +31 10 4391031.
E-mail address: m.vandenbent@erasmusmc.nl (M.J. van den Bent).
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Table 1 – Median, 1- and 2-year survival after various postoperative adjuvant treatment in high-grade glioma, in bold the outcome of EORTC study 26981

Reference	Treatment	Median survival	1-year survival (%)	2-year survival (%)
Walker 1978[1]	Supportive care	3 mo	3	0
	BCNU	4 mo	12	0
	RT	8 mo	24	1
	RT + BCNU	8 mo	32	5
Glioma Meta-analysis Trialists Group[3]	RT		40	15
	RT + CTX		46	20
EORTC 26981[4]	**RT**	**12**	**51**	**10**
	RT + temozolomide	**15**	**61**	**27**

RT, radiotherapy; CTX, chemotherapy; SRS, stereotactic radiosurgery; BCNU, carmustine; mo, months.

will summarize the current knowledge in the field, and aims to highlight some novel developments and directions for future research.

2. Temozolomide: pharmacology and mechanisms of action

TMZ is an oral alkylating agent, which is rapidly (peak concentrations after 30–90 min) absorbed, penetrates into all body tissues including the central-nervous system and spontaneously converts into the active metabolite MTIC.[5] Although recommended to be taken in fasting state, food will have little influence with a 10% reduced AUC and a delayed peak concentration.[6] Similar to nitrosoureas, TMZ acts as a DNA alkylating agent. Alkylation of the O6-position of guanine is one among many DNA adducts formed, but it is of major importance for the induction of mutations and the cytotoxic action of these drugs. During subsequent DNA replication, the methylated guanine could be shown to be paired with thymidine instead of cytidine, which initiates futile mismatch repair and eventually may induce apoptosis. Cells have the capacity of restoring guanine through the DNA excision repair enzyme O^6-methylguanine–DNA methyltransferase (MGMT), also known as O^6-alkyl-guanine–alkyltransferase (AGAT) (reviewed by Gerson[7]). During this saturable process the enzyme is consumed. High endogenous MGMT activity in cancer cells blunts the treatment-effect of alkylating agent chemotherapies creating a resistant phenotype and may be an important determinant of treatment failure.[8–12] Silencing the *MGMT* gene by promoter methylation that impairs expression of the DNA repair enzyme has been associated with prolonged survival in glioma patients treated with nitrosoureas or with TMZ.[13,14]

3. Temozolomide in recurrent glioma

TMZ has demonstrated its activity initially in patients with recurrent high-grade glioma. Three pivotal phase II studies with identical entry criteria were conducted for patients with GBM and with anaplastic astrocytoma (AA). Despite disappointingly low objective response rates in GBM of 5% and 7%, respectively, but with an interesting response rate of 35% in the AA trial, these studies suggested an increase in the fraction of patients being progression-free at 6 months compared to a historical database.[15–17] Subgroup analysis suggested that mainly patients who had not received prior adjuvant chemotherapy benefited from this treatment.

4. Temozolomide schedules

In vitro and phase I studies have established the initial standard dosing of TMZ (150–200 mg/m^2/day) for 5 days every 4 weeks. Novel continuous administration schedules have since been developed, allowing for more intensive and dose-dense regimens. Using these low-dose but daily schedules, the actual dose-intensities could be increased up to over 2-fold (e.g., one week on/one week off, three weeks on/one week off, 42 days every 60 days).[18–20] The protracted administration of temozolomide, even at relatively low daily doses, was shown to lead to significant and prolonged depletion of MGMT activity.[21] Although this may potentially enhance the antitumour activity of the agent, to date there are no conclusive data available demonstrating that these more dose-dense regimens offer clinically relevant increased anti tumour activity.[22,23] No randomized studies exist, and the reported improved outcome in terms of 6 months progression-free survival may be a matter of patient selection. One novel adverse effect of the continuous temozolomide administration schedules is induction of profound lymphocytopenia with low CD4 counts.[24] The common use of corticosteroids in brain tumour patients adds further to an immunodeficient state, predisposing patients to opportunistic infections. Increased incidence of opportunistic infections, in particular pneumocystis carinii pneumonia and Kaposi sarcoma, has been reported.[25,26] Monitoring of CD4 counts and antibiotic prophylaxis with trimethroprim/sulfametoxazole or pentamidine inhalations for patients at risk have been proposed.

5. Temozolomide for patients with newly diagnosed glioblastoma

Based on the activity in recurrent glioma and in vitro data suggesting additive or supra-additive activity when TMZ was administered concomitantly with radiotherapy, a phase II trial investigated the administration of low-dose TMZ daily (75 mg/m^2 daily 7/7 days week) with concomitant radiotherapy for up to 7 weeks, followed by six cycles of adjuvant TMZ (5-days q28 days). This treatment was shown to be feasible and well

tolerated.[26] The European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) Clinical Trials Group compared this new combined modality regimen to standard radiotherapy alone in a large prospective randomized phase III trial on 573 patients.[4] Primary trial endpoint was survival. This study unequivocally demonstrated that the combination of TMZ and radiotherapy (RT) followed by up to six cycles of adjuvant TMZ improves survival. With combined modality treatment, the 2-year survival increased from 10% to 26% (Table 1). Subgroup analyses suggested that patients of all age groups (up to age 70 allowed in the trial) and independent of prior tumour resection benefited from this treatment. As in other fields of oncology, patients with a modest performance status did not benefit from the addition of chemotherapy. Overall, the combined treatment was well tolerated, and the main reason for early discontinuation was disease progression.

The clinical relevance of the mechanistic implication of the DNA repair enzyme MGMT in alkylating chemotherapy was tested in this randomized EORTC/NCIC trial. Samples from 206 patients could be analysed for the status of the *MGMT* gene promoter using methylation-specific PCR (MSP).[27] In 45% of tumour samples, the *MGMT* gene promoter was methylated, and the gene was silenced. Overall, patients with a silenced *MGMT* gene had longer survival. Break down of the data by treatment strongly suggests that the *MGMT* methylation status is a predictive marker for benefit from TMZ chemotherapy. For patients in the TMZ/RT arm, the 2-year survival rate was 46% when their tumour presented a methylated *MGMT* status in contrast to only 14% in patients with an unmethylated *MGMT* gene promoter (Table 2). Thus, in this molecularly defined subgroup the TMZ is even more effective increasing the median survival by 9 months to 21.7, while patients with an unmethylated *MGMT* gene promoter had little, if any, TMZ-derived benefit with a median survival of 12.7 months.

Confirmation of these results in a second trial would then suggest that only patients whose tumours comprise a methylated *MGMT* promoter (thus silenced gene) should receive TMZ, while for other patients, alternative strategies must be considered. Strategies aiming at overcoming MGMT-mediated resistance are currently developed. Administration of agents like O6-benzyl-guanine, a non-cytotoxic substrate of MGMT, or in combination with nitrosoureas is limited by increased systemic toxicity, mainly myelosuppression. Continuous administration of temozolomide has been shown to deplete intracellular MGMT and novel dose-dense schedules are currently explored as adjuvant treatment in large randomized trials.

Current phase II and phase III studies are investigating whether the addition of agents targeting the EGF receptor pathway (e.g., the tyrosin kinase inhibitor erlotinib), VEGF and PDGF receptors (e.g., PTK787) or the tumour vasculature (e.g., the alphaVbeta3 inhibitor cilengitide) to the combination of TMZ and radiotherapy further improve survival in newly diagnosed GBM.

6. Temozolomide in elderly

The first randomized studies on anaplastic glioma have clearly shown the superiority of radiotherapy over chemotherapy.[1] However, in elderly patients with a particularly dismal prognosis, simple and least toxic therapies are required. Encouraged by reports of high response and disease stabilization rates with TMZ chemotherapy in previously untreated GBM patients,[28] chemotherapy in lieu of radiotherapy has been evaluated in a limited number of patients in uncontrolled trials.[29] Chinot and colleagues reported a median survival of 6.4 month in a prospective study on 32 patients.[29] Glantz and colleagues observed in a non-randomized study a survival outcome after treatment with temozolomide ($n = 32$) that was at least as good as 'historical controls' treated with radiotherapy in the same time period within the same institution.[30] Furthermore, both groups were comparable with respect to known prognostic factors. The treatment outcome of these two studies appears similar compared to the results of radiotherapy only in elderly or poor prognosis GBM patients.[31,32] In both studies toxicity was modest, although in one study dose delays and dose reductions were needed in 38% and 13% of patients. This underlines that a more cautious approach to chemotherapy is needed in elderly patients. Both studies give support for further investigations into this approach. The NCIC together with the EORTC are currently planning a randomized trial comparing standard hypofractionated radiotherapy with TMZ added to the same RT, or TMZ alone.

7. Temozolomide in combination with other cytotoxic agents

In the past years results, of several novel combinations of alkylating agents with drugs with a different mode of action investigated in recurrent GBM have been published: cisplatin and TMZ,[33] marimastat and TMZ,[34] cisretinoic acid and TMZ,[35] thalidomide and carmustine[36] (Table 3). Each of these combinations report high response rates and favourable percentages of patients free from progression at six months. Invariably, the authors conclude the results are promising, and then move on to the next combination. In the absence

Table 2 – Median survival and 2-year survival in EORTC study 26981 in relation to the methylation status of the MGMT promoter[27]

Treatment	Median survival (months)		Two year survival (%)	
	Radiotherapy[a]	Radiotherapy plus temozolomide	Radiotherapy[a]	Radiotherapy plus temozolomide
Unmethylated *MGMT* promotor	11.8	12.7	<2	13.8
Methylated *MGMT* promotor	15.3	21.7	22.7	46

a 72% of the patients in the radiotherapy-arm received alkylating chemotherapy at recurrence.

Table 3 – A summary of recent studies on recurrent glioblastioma multiforme, except for *, recurrent anaplastic astrocytoma

	Drugs	*n*	CR + PR (%)	6 mo PFS (%)
Yung et al.[16]	TMZ 150–200 mg/m^2 day 1–5 every 4 weeks	112	5.4	21
	Procarbazine	113	5.3	8
Yung[15*]	TMZ 150–200 mg/m^2 day 1–5 every 4 weeks	162	35	46
Jaeckle[35]	TMZ 150–200 mg/m^2 day 1–5 every 4 weeks + *cis* retinoic acid	40	4.5	32
Groves[34]	TMZ 150–200 mg/m^2 day 1–5 every 4 weeks + marimastat	44	13.6	39
Fine[36]	BCNU 200 mg/m^2 + 800–1200 mg thalidomide every 6 weeks	40	24	27
Brandes[33]	TMZ 200 mg/m^2 day 1–5 + cisplatin 75 mg/m^2 every 4 weeks	50	20	34
Wick[23]	TMZ 150 mg/m^2 one week on/one week off	21	10	43
Khan[22]	TMZ 75 mg/m^2 daily for 6 weeks in 10 week cycles	28	0	19
Prados[41]	BCNU 150 mg/m^2 + TMZ single dose 550 mg/m^2	36	5.5	21
Brandes[61]	BCNU 80 mg/m^2 day 1–3	40	15	17.5
Brandes[62]	BCNU 100 mg/m^2 day 1 + 175 mg/m^2 CPT-11 weekly for 4 weeks in cycles of 6 weeks	42	21.4	30
Kappele[63]	PCV	63	11	29

Abbreviations: *n*, number of patients; CR, complete response; PR, partial response; PFS, progression-free survival; BCNU, curmustine; TMZ, temozolomide; PCV, procarbazine, CCNU and vincristine.

of randomized trials, no conclusion can be drawn from these studies and results may solely reflect patient selection.

Because of the role of MGMT in the resistance to alkylating chemotherapy, several studies added a MGMT depleting agent in order to increase efficacy. For this purpose, both drugs with intrinsic cytotoxic activity as well as drugs that only deplete MGMT (O6-benzylguanine; O6-BG) were used. As one might expect, such combinations also increased toxicity and necessitated the use of reduced dosages as compared to the same agents when given as single agent treatment.[37,38] First results of this strategy showed that adding O6-BG to carmustine did not induce regression in carmustine resistant gliomas, although a similar trial in temozolomide pretreated patients showed some evidence of benefit by adding O6-BG to TMZ.[39,40] A trial exploring the combination BCNU and temozolomide concluded however that the outcome of the combination appeared of similar efficacy as single agent temozolomide (Table 3).[41]

8. Irinotecan

Since its initial report of activity (of irinotecan CPT11) in recurrent malignant glioma (17% objective response rate in GBM), this drug was further explored in several single agent studies.[42–44] These did not confirm the high response rates initially reported. Currently combinational regimens are being investigated, in particular of temozolomide and irinotecan. A study investigating the combination, BCNU and irinotecan, yielded a response rate similar to other combination studies with temozolomide. As irinotecan is mainly metabolized in the liver, dependent on the P450 system, it can only be reliably administered to patients not using hepatic enzyme inducing antieptileptic drugs (EIAED).

9. Oligodendroglioma

Oligodendroglioma and to a lesser extent, oligoastrocytoma, are responsive to chemotherapy. Two-thirds of patients with recurrent tumours were shown to respond to PCV chemotherapy, consisting of procarbazine, CCNU and vincristine.[45,46] The recent finding of a high percentage of these tumours with a methylated *MGMT* status (>80%) may in part explain this clinical feature.[47] Further molecular studies showed that in particular, patients with oligodendrogliomas with loss of heterozygosity on both, the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q), are very sensitive to PCV chemotherapy: almost all patients responded to PCV chemotherapy.[48,49] The 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 and oligoastrocytoma.[50,51] High response rates of 53% (26% complete responses) and 25% were observed to first and second-line temozolomide chemotherapy, respectively. Genetic analysis confirmed the importance of 1p/19q loss for chemotherapy response also for TMZ.[52] The currently available data suggest that about 55–65% of patients with recurrent oligodendroglioma respond to first-line chemotherapy, but in second-line the response rate dropped to 20–25% regardless of the chosen sequence of treatment: temozolomide first or PCV first.

Both the RTOG and the EORTC investigated the addition of PCV chemotherapy to radiotherapy in newly diagnosed anaplastic oligodendroglioma.[53] The RTOG study used neoadjuvant with a dose intensified PCV schedule, whereas the EORTC study used classical adjuvant design with standard PCV; in both studies the control arm received radiotherapy only (but further treatment at the time of progression was left to the discretion of the treating physician). Most patients received further chemotherapy at the time of progression. Although both studies observed an increase in progression-free survival in the PCV arm, this did not translate in to an increase in overall survival. Patients with 1p/19q loss had a clearly better outcome (median survival over 6–7 years as compared to 2–3 years in patients without 1p/19q loss), but the improved outcome was regardless of treatment. Both studies showed, that in this chemosensitive tumour, the timing of chemotherapy was not relevant (at first diagnosis or at recurrence), as long as it was given.

The somewhat counter-intuitive result of three large studies on adjuvant treatment in glioma is that the EORTC study, on concurrent and adjuvant temozolomide in chemoresistant GBM, resulted in survival benefit, whereas a similar EORTC and RTOG trials on chemosensitive oligodendroglioma failed to provide survival benefit. Perhaps the most significant difference between these trials is the combination of radiotherapy with daily temozolomide in the GBM study. It may well be that this part of the treatment is the essential part for the improved outcome, but this must be proven in new trials.

Despite the absence of a formal trial many clinicians today propose upfront temozolomide TMZ as first treatment in 1p/19q loss oligodendroglioma. This is clearly an experimental approach, based on the assumption that RT is likely to induce delayed cognitive deficits. This assumption is questionable though if modern RT techniques are used with dose fractions of less than 2 Gy.[54] Eventually, this may be more a matter of side-effects, and one should realize that especially for limited size lesions, RT offers an effective treatment of short duration (6 weeks instead of 12–24 months of chemotherapy).

10. Low-grade glioma

Several recently published studies have explored upfront treatment of newly diagnosed low-grade glioma (in particular oligodendroglioma) with chemotherapy with either PCV or temozolomide.[55–58] These trials show that objective responses are rare, despite prolonged disease stabilization in the majority of patients. Measuring response in low-grade tumours in the absence of contrast enhancement may be particularly difficult. On MRI abnormalities on T2 weighted images are unlikely to decrease significantly, and functional imaging by positron-emission tomography using amino-acid tracers is not widely available.[56] For clinical trials, time to progression may be the preferred endpoint.[59,60]

Whether there is truly an advantage in treating these patients with upfront chemotherapy compared to initial RT is currently the subject of a randomized EORTC/NCIC phase III trial. Outside trials, it is questionable whether upfront chemotherapy should be given to low-grade glioma (LGG) patients, even in case of oligodendroglioma with 1p/19q LOH. Radiotherapy is also a very effective and proven treatment, and in the end, the choice of therapy may well be a matter of acute and long-term toxicity based on individual patient factors and tumour size and location. Whether a short course of radiotherapy (typically 6 weeks) should be replaced by prolonged (proposed duration 1 year) and costly administration of chemotherapy needs to be proven. Possibly the sequence of treatment modalities is of lesser or no importance in overall survival. Although the addition of TMZ to radiotherapy has been proven superior in the treatment of aggressive glioblastoma, extending this approach to low-grade glioma is potentially harmful. In a disease with median survival rates of 5–7 years, with a significant proportion of patients living 10–15 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 the maximum use of all therapeutic arms upfront.

11. Conclusions and outlook

Temozolomide has become the mainstay of chemotherapeutic treatment of high-grade glioma. The use of a cytotoxic agent with the capacity of crossing the blood–brain barrier in combination with radiotherapy has a clear impact on progression and survival of glioblastoma patients. Molecular studies have shown that specific tumour characteristics like a silenced *MGMT* gene may allow tailoring treatment for individual patients. New trials are planned to confirm these findings, but also to develop strategies allowing depletion of MGMT in unmethylated tumours, e.g., by using more intensive treatment schedules, but also to evaluate the role adjuvant treatment after the completion of concurrent chemoirradiation.

The current progress in the treatment of malignant glioma should fuel interest in further improving the outcome of patients with brain tumours. Optimizing current agents and treatment regimens, combination with new cytotoxic and targeted molecules hold promise. The close interaction and integration of laboratory findings and clinical research will allow to identifying new treatment targets and are 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 tumours 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 for better understanding of successes and failures.

Conflict of interest statement

None declared.

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