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Reviews

The role of cytotoxic drugs in the treatment of central nervous system gliomas

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Abstract

Gliomas are the most frequent subtype of primary brain tumors. They are lethal tumors, characterized by diffuse infiltration of the brain and a high resistance to conventional cancer therapies. Following maximal neurosurgical resection, bound to the limits of acceptable neurological sequelae, immediate post-operative radiotherapy is indicated in the majority of patients. Chemotherapy with the alkylating agent temozolomide, administered daily concomitantly to radiotherapy, and followed by six adjuvant monthly cycles, significantly improves the survival of newly diagnosed glioblastoma patients and has become the standard of care. Temozolomide is also the most often used chemotherapeutic treatment for recurrent low-grade and anaplastic gliomas after initial surgery and irradiation. The potential role of post-operative temozolomide in the first line treatment for low-grade and anaplastic glioma is currently under investigation in phase III trials. After failure of temozolomide, there is only limited activity of any other cytotoxic agent and the benefit of such second line therapy seems to be limited to a small subgroup of patients with the most chemosensitive gliomas. Abnormal hypermethylation of the promoter of the MGMT gene has been correlated with the response of glioma to alkylating chemotherapy. The loss of chromosomal arms 1p and 19q are genetic markers characteristic for gliomas with oligodendroglial differentiation which are also most sensitive to treatment. The predictive and prognostic value of these molecular markers is currently being determined prospectively in phase III studies. Anti-angiogenic agents and targeted receptor tyrosine kinase inhibitors are new pharmacological classes with activity against malignant gliomas. Phase III clinical studies evaluating combinations of these new agents with classical cytotoxic agents in first and in second line have recently been initiated.

Key words: Chemotherapy; glioblastoma; temozolomide; nitrosurea; MGMT.

Introduction

Gliomas are the most common tumors of the central nervous system. The annual incidence of malignant gliomas is estimated at 5 cases per 100 000 persons worldwide without striking differences between geographic regions or ethnicities (DeAngelis, 2001; Wen *et al.*, 2008). Each year approximately 700 new cases are diagnosed in Belgium (http://www.kankerregister.org/). Gliomas are classified by the World Health Organization (World Health Organization, WHO) according to their histological features and degree of differentiation (Louis *et al.*, 2007). The largest subgroups are glioblastomas (WHO grade 4 glioma, 60-70% of all cases), anaplastic gliomas (WHO grade 3, 20-30% of all cases) and low grade gliomas (WHO grade 2). Histopathologically, WHO grade 2 and 3 gliomas can be further divided into astrocytomas, mixed oligoastrocytomas and oligodendrogliomas. WHO grade 1 gliomas are a separate group of non infiltrating tumors that can be treated with curative-intent surgery or radiation therapy in most cases. WHO grade 2-4 gliomas, by contrast, are cancers that diffusely infiltrate the normal brain, and are not amenable for curative surgical removal. Because of their marked resistance to adjuvant treatments such as radiation therapy and chemotherapy, gliomas have a high mortality rate that nearly equals their incidence rate (http://www.kankerregister.org/; Cancer Incidence and Survival in Flanders, 2000-2001). The age-specific survival of patients with primary malignant brain tumors shows a relatively uniform survival in patients aged more than 65 years at diagnosis, but there are more marked intercountry differences in younger patients (Sant *et al.*, 1998).

Chemotherapy (cytotoxic agents)

Glioblastomas and anaplastic astrocytomas belong to the most chemoresistant human tumors. This can in part be explained by the blood-brain barrier, which largely prevents the penetration of non-lipophilic drugs or drugs of high molecular size in the central nervous system, and by the intrinsic resistance mechanisms of these tumors. The DNA alkylating cytotoxic agents temozolomide and the nitrosureas (e.g. nimustine (ACNU), carmustine (BCNU), lomustine (CCNU) and fotemustine) are the most active chemotherapeutics. Temozolomide is the only cytotoxic agent for which a gain in overall survival has been demonstrated when added to postoperative radiation therapy, as compared to radiotherapy alone for patients with newly diagnosed glioblastoma.

Until the nineties, nitrosurea and procarbazine, and the combination regimen PCV (procarbazine, CCNU and vincristine) were the most studied chemotherapeutics in the treatment of glioma. Individual randomized trials for adjuvant treatment after surgery and irradiation in first or in second line, however, were inconclusive with respect to a possible survival benefit. A systematic review and meta-analysis of 3004 patients from 12 randomized trials showed a limited improvement of the 1-year survival of 6% (95% CI 3-9) with prolongation of median survival (Stewart, 2002). The largest randomized study comparing adjuvant PCV versus radiation-only (conducted by the Medical Research Council Brain Tumor Working Party between September 1988 and May 1997), did not show a statistically significant benefit on the median survival after the addition of chemotherapy (the median survival was 9.5 months for RT and 10 months for the RT-PCV (log-rank P = 0,50) (2001).

In view of the potentially severe toxicity of the nitrosurea (cumulative myelosuppression, lung- and liver toxicity), the systematic use of chemotherapy remained controversial and was not accepted as a standard of care for patients with high-grade gliomas.

Although no significant survival benefit could be shown for the nitrosurea BCNU (alone) and CCNU (in combination) in randomized trials in first line, these substances are still used as a second line treatment option. A phase II trial conducted in the nineties reported a time to progression of 13.3 weeks and a progression-free survival rate at 6 months of 17.5% in patients with recurrent glioblastoma following surgery and radiation therapy (Brandes *et al.*, 2004).

A number of other agents (e.g. procarbazine, irinotecan, cisplatinum, carboplatinum, hydroxyurea, etoposide) have only demonstrated marginal activity with variable response rates in different small clinical studies, not reaching a threshold for defining a clear role for them in the care for glioma patients (Franceschi *et al.*, 2004; Fulton *et al.*, 1996; Korones *et al.*, 2003; Levin, 1992; Vredenburgh *et al.*, 2009; Yung *et al.*, 2000).

Chemotherapy for malignant gliomas was not integrated into the standard of care before the late nineties. The first breakthrough was the observation that anaplastic oligodendrogliomas and oligoastrocytomas (WHO grade III) responded markedly better to nitrosurea-based PCV chemotherapy than astrocytomas of the same grade of malignancy. Associated with the oligodendroglial differentiation, the combined loss of chromosome arms 1p and 19q proved to be a genetic marker predicting the sensitivity to chemotherapy more specifically than conventional histology (Cairncross *et al.*, 1998; Michotte *et al.*, 2004). Although PCV initially appeared to be specifically active against oligodendroglial tumors, reports on a comparable activity of temozolomide indicated that oligodendroglial gliomas with 1p and 19q chromosomal loss are in general more chemosensitive (Blakeley *et al.*, 2008; Mikkelsen *et al.*, 2009; Smith *et al.*, 2000; Vogelbaum *et al.*, 2009).

The loss of genetic material at chromosome 1p and 19q results from an unbalanced translocation t (1; 19) (q10, p10) (Jenkins *et al.*, 2006). Based on the results from uncontrolled studies, two randomized phase III trials were conducted in patients treated with post-operative radiotherapy with or without concomitant PCV for anaplastic oligodendroglioma and anaplastic oligoastrocytoma. PCV was either administered before (neoadjuvant) or after radiotherapy (adjuvant) in the experimental arm of the RTOG 94-02 and EORTC 26951 studies. In both studies, the progression-free survival (PFS) was longer in the PCV plus RT arm (median PFS was 2.6 versus 1.9 years ($p = 0.053$) and 23 versus 13.2 months ($p = 0.0018$) for PCV plus RT versus RT in the RTOG 94-02 and EORTC 26951 study, respectively). However, this did not result in a statistically significant overall survival benefit (median survival was 4.8 years versus 4.5 years ($p = 0.830$) in the RTOG 94-02 study and 40.3 versus 30.6 months ($p = 0.23$) for PCV plus RT versus RT in the RTOG 94-02 and EORTC 26951 study, respectively). In both studies, patients with progressive disease pre-treated with radiotherapy only, could receive PCV treatment for recurrence (this was the case in 82% of the patients in the EORTC study). The safety assessment confirmed that PCV chemotherapy is associated with a therapy-limiting

toxicity. A significant percentage of patients discontinued PVC-treatment before trial completion (38% of patients in the EORTC study discontinued for toxicity reasons). In both studies, a systematic analysis was performed for the loss of chromosome arms 1p and 19q in the tumor cells. The predictive value of these molecular markers was confirmed in both studies. Patients with combined 1p and 19q loss (25% of the entire study population) had a longer survival than those with an isolated loss of 1p, who still had a better survival than patients with an isolated loss of 19q. Patients without 1p and 19q loss had the worst survival. In none of these genetically identified subgroups, a significantly improved survival could be demonstrated after addition of PCV. Due to the low mortality rate in the 1p/19q subgroup at the time of the survival analyses, however, a possible benefit of adding PCV could not be excluded. At present, both studies do not provide sufficient evidence for adding PCV chemotherapy to initial treatment of patients with anaplastic oligodendroglioma or oligoastrocytoma. A later analysis with additional follow-up of the survival in both these studies will be of interest to verify whether or not a trend can be found for the subgroup of patients with the longest survival to benefit from PCV-chemotherapy.

Temozolomide

Temozolomide is a cytotoxic agent (second generation imidozotetrazine derivative) with an excellent oral biodisponibility (Beale *et al.*, 1999). In contrast to dacarbazine (DTIC), temozolomide is not activated by hepatic metabolization, but undergoes spontaneous degradation at physiological pH to form the cytotoxic metabolite 5 - (3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC). The main cytotoxic activity of MTIC is mediated by DNA alkylation at position O6 of guanine with additional alkylation at the N3 and N7 positions. The cytotoxicity of temozolomide is dose- and regime-dependent in preclinical experiments. Therefore, the drug was initially developed as a once-daily oral administration for 5 consecutive days every 28 days (5/28d temozolomide regimen) (Brada *et al.*, 1999; Newlands *et al.*, 1992). The daily dose for this schedule was set at 150 mg/m^2/day for a first cycle, with an increase to 200 mg/m^2/day for subsequent cycles when no significant toxicity occurs during the first cycle. The efficacy and toxicity of this schedule for the treatment of patients with recurrent gliomas is well documented in several phase II trials (Bower *et al.*, 1997; Brada *et al.*, 2001; Brandes *et al.*, 2001; Janinis *et al.*, 2000; Middleton *et al.*, 2000; Newlands *et al.*, 1996; Osoba *et al.*, 2000; Trent *et al.*, 2002; Yung *et al.*, 2000). Activity of temozolomide has also been reported in the first-line treatment of patients with low-grade (WHO grade II) glioma and for recurrent low-grade glioma following radiation therapy (Brada *et al.*, 2003; Hoang-Xuan *et al.*, 2004; Neyns *et al.*, 2005; Pace *et al.*, 2003; Quinn *et al.*, 2003).

Nausea and vomiting are the most common side effects of the 5/28d temozolomide schedule, but can be controlled with standard anti-emetics. Myelosuppression is the dose-limiting toxicity (mainly grade 3/4 thrombocytopenia and – to a lesser degree – neutropenia). Grade 3 or 4 thrombo-and/or neutropenia occurs in 8 to 17% of the patients treated with temozolomide (both in the prospective phase II as in observational trials) (Chang *et al.*, 2004; Everaert *et al.*, 2004). Severe toxicity typically occurs during the first two cycles of treatment and temozolomide has no known cumulative toxicity with this regimen (in contrast to nitrosurea). Myelosuppression usually occurs late in the cycle (day 21) with normalization of haematological values during the subsequent 2 to 4 weeks. Serious and unpredictable myelosuppression, leading to discontinuation of temozolomide, is very rare but has been reported (Nagane *et al.*, 2008).

In patients with a first recurrence after surgery and radiotherapy, alone or combined with adjuvant nitrosurea-based chemotherapy, the activity of the temozolomide 5/28d regimen is strongly related to the initial WHO-grade of malignancy. In a phase II pilot study for patients with recurrent anaplastic astrocytomas (AA) or anaplastic oligoastrocytomas (AOA), the 6-month progression-free survival (PFS) was 48% (95% confidence interval, 39%-58%). In patients with eligible histology (AA an AOA), the overall survival (OS) was 14.5 months. In 35% of the patients a tumor response (according to the Macdonald criteria) was documented (34% for AA and 43% for AOA patients) (Fig. 1 and Table 1). In patients with recurrent anaplastic oligodendroglioma, 43.8% responded (16.7% CR + 27.1% PR) and 39.6% had tumor stabilization. Median PFS was 6.7 months and median OS was 10 months (Chinot, 2001). A lower activity was noted in patients with recurrent glioblastoma (objective tumor response in 5.4-8% and PFS at 6 months 18-21%) (Table 1) (Brada *et al.*, 2001; Yung *et al.*, 2000). Despite the limited activity in patients with recurrent glioblastoma, temozolomide was superior to procarbazine (Yung *et al.*, 2000) More important, treatment with temozolomide resulted in an improved health-related quality of life score (HRQL), as compared to procarbazine (Osoba *et al.*, 2000).

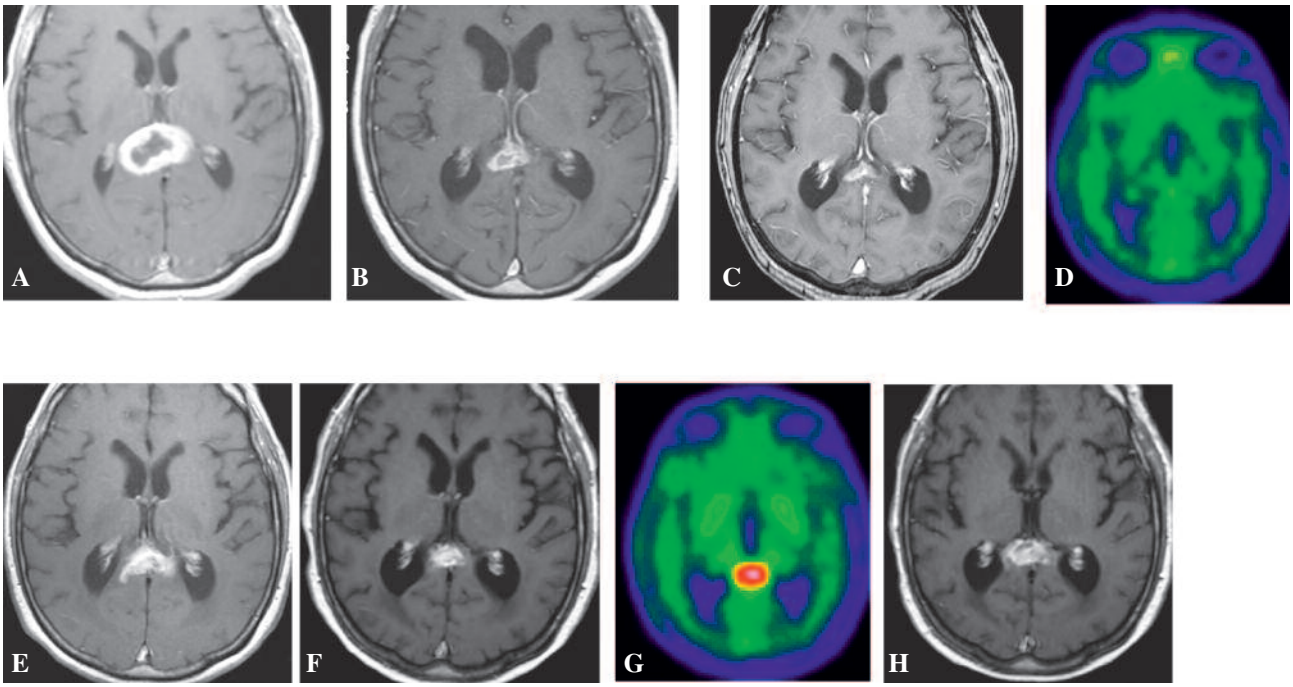

FIG. 1. — Following the occurrence of problems with her equilibrium and episodes of confusion, this 68 year old female patient was diagnosed in March 2007 with a gadolinium enhancing tumor located in the splenium corpus callosum (A). A stereotactic biopsy revealed glioblastoma without EGFR gene amplification. Treatment consisted of fractionated radiation therapy (30×2 Gy) with concomitant daily temozolomide (75 mg/m^2/day) followed by 6 adjuvant cycles of 5 out of 28 day temozolomide (administered at a daily dose of 200 mg/m^2). Treatment was well tolerated and sequential MRI of the brain showed tumour regression. The residual gadolinium enhancing mass at the end of temozolomide therapy (B) continued to shrink during the following 5 months (C) and no uptake was revealed on PET imaging with an amino-acid tracer (fluorinated fenyl-methyl-alanine, FMP) (D). Nine months after the end of adjuvant temozolomide, progression of the glioblastoma was observed along with recurrent clinical symptoms (E). Temozolomide was reinitiated (5 out of 28 day regimen at) and resulted again in radiologic regression with symptomatic improvement (F). However, at the time of best radiological response, following 8 cycles of temozolomide, persistant uptake of FMP was still documented on PET (G). After 10 treatment cycles, progression was documented under temozolomide treatment.

Concomitant radiotherapy and temozolomide

The main benefit of temozolomide in the treatment of glioblastoma was demonstrated by adding daily temozolomide (at a dose of 75 mg/m^2/day) to post-operative radiotherapy, followed, after a 4 weeks therapy-free interval, by another 6 cycles of adjuvant temozolomide (administered 5 out of 28 days at a dose of 150-200 mg/m^2/day). The efficacy of this regimen was demonstrated in a randomized phase III study conducted by the EORTC and NCIC (26981-22981/CE.3). This combination therapy significantly improved survival compared with radiotherapy alone (hazard ratio 0.6, 95% CI 0.5-0.7; $p < 0.0001$) (Stupp *et al.*, 2005). Recently, an update of the survival results with a median follow-up of more than 5 years was reported (Table 2) (Stupp *et al.*, 2009).

The toxicity of this combined treatment was acceptable (7% experienced a grade 3 or 4 toxicity) and the health-related quality of life (HRQOL) was not affected (Taphoorn *et al.*, 2005). In the preceding phase II trial, pneumocystis-carinii pneumonia (PCP) infections were seen in some patients during the concomitant RT/TMZ phase (Stupp *et al.*, 2002). These were probably provoked by a frequent treatment-related lymphopenia and the corresponding immunosuppression. In the phase III trial, PCP prophylaxis was mandatory (eg trimethoprim/methoxazole forte 3×1 tablet per week) during the concomitant treatment phase. As a result, no additional cases of PCP or other opportunistic infections were found (Stupp *et al.*, 2005).

After completion of the EORTC/NCIC 26981-2298/CE study, significant prognostic factors could be identified in the combined modality treatment group. Using Recursive Partitioning Analysis (RPA), patients were attributed to different prognostic groups, with the following variables: age, WHO performance status, extent of surgical resection, and the Mini Mental State. The three-year survival rates according to these RPA classes are shown in Table 3 (Stupp *et al.*, 2009).

Table 1

Results of 3 pivotal phase II studies with temozolomide for a first recurrence of glioblastoma or anaplastic (oligo)astrocytoma

Study	Patients: number and indication	Treatment	Number of patient with preliminary treatment with nitrosurea regimens	Best objective tumor respons (BOR, %)	Disease control (DCR, %)	6-months PFS (%)	6-months OS (%)
Yung *et al.* (Yung, Prados *et al.*, 1999)	162 AA/AOA	TMZ 150–200 mg/m²/d X5d q28d	60	35	61	46	75
Brada *et al.* (Brada, Hoang-Xuan *et al.*, 2001)	138 GBM		29	8	51	18	46
Yung WK *et al.* (Yung, Albright *et al.*, 2000)	225 GBM		65	5.4	45	21[2]	60[3]
		PCB 125-150 mg/m²/d x28d q56d	68	5.3	32	8[2]	44[3]

[1] All patients received preliminary treatment with radiotherapy; [2] p = 0.008; [3] p = P = 0.019; GBM = glioblastoma.

Table 2

Survival rate after a median follow up of 61 months in the EORTC/NCIC 26981-22981/CE.3 phase III study (Stupp, Hegi *et al.*, 2009)

Percentages (%)	OS		PFS	
	RT n = 286 % (95% CI)	RT + TMZ n = 287 % (95% CI)	RT n = 286 %	RT + TMZ n = 287 %
2-year	10.9 (7.6-14.8)	27.2 (22.2-32.5)	1.8	11.2
3- year	4.4 (2.4-7.2)	16.4 (12.0-20.6)	1.3	6
4- year	3.0 (1.4-5.7)	12.1 (8.5-16.4)	1.3	5.6
5- year	1.9 (0.6-4.4)	9.8 (6.4-14.0)	NR	NR
Hazard ratio	0.63 [0.53-0.75] *P* < 0.0001		0.56 [0.47-0.66] P < 0.0001	

NR: not mentioned; OS: overall survival; PFS: progression-free interval.

MGMT promoter methylation status

The cytotoxic effects of temozolomide and other alkylating chemotherapeutic agents like nitrosureas are counteracted by the activity of several cellular DNA repair mechanisms, including the O6-alkylguanine-DNA alkyltransferase ((A) GAT or MGMT), the mismatch repair and the base excision repair (Sarkaria *et al.*, 2008). The MGMT repair protein restores DNA damage at the O6 position, and is considered to be the main resistance mechanism against the cytotoxic effect of temozolomide. After application of alkylating agents, DNA adducts on the O6 position of guanine (O6MeG) and causes an inappropriate binding with thymine during the following replication cycle. This abnormal base linkage in the DNA structure is recognized by the MSH2-MSH6 dimer of the mismatch repair system (Cahill *et al.*, 2007; Hegi *et al.*, 2008; Hunter *et al.*, 2006; Sasaki *et al.*, 2001). In the absence of the MGMT repair protein, cells will repeat insufficient repair cycles, and finally undergo apoptosis (Lefranc *et al.*, 2007). The mechanisms leading to temozolomide resistance in the absence of MGMT are only partially clarified.

Table 3

Three-year survival percentages according EORTC RPA prognostic groups from the EORTC/NCIC 26981-22981/CE.3 phase III study (Mirimanoff, Gorlia *et al.*, 2006)

EORTC RPA groups		3-year survival-%		Hazard ratio
		RT	RT/TMZ	
III	Age < 50, WHO PS 0 – 1	10.3	31.5	0.54 [0.33-0.88] P = 0.012
IV	Age < 50, WHO PS 2 or Age ≥ 50, MMSE ≥ 27 and resection	4.1	15.8	0.62 [0.49-0.79] P = 0.0001
V	Age ≥ 50, MMSE < 27, and biopsy only	2.1	10	0.69 [0.52-0.93] P = 0.014

One known mechanism is the loss of mismatch repair protein MSH6 (Cahill *et al.*, 2007; Hunter *et al.*, 2006).

In vitro, a strong relationship has been seen between high cellular reserves of MGMT protein and resistance to temozolomide. Depletion of MGMT results in an increased sensitivity to temozolomide (Baer *et al.*, 1993; Wedge *et al.*, 1996). Thirty to 40% of high-grade gliomas have a hypermethylated promoter of the MGMT gene (this is an abnormal epigenetic modification of DNA occurring in malignant cells). This results in an arrest of MGMT gene transcription and deficient MGMT repair protein synthesis. MGMT promoter hypermethylation is detectable by using a methylation-specific polymerase chain-reaction assay (MSP), and is correlated with an increased rate of tumor response and improved survival of glioma patients treated with alkylating agents. A quantitative real-time MSP assay for the measurement of MGMT promoter methylation (developed by Oncomethylome Science) is currently being used in three phase III trials (RTOG0525, EORTC protocol 22033–26033 and EORTC protocol 26053-22054). The results obtained from these studies will determine whether MGMT-promoter status should be implemented as a predictive test for the guidance of temozolomide treatment in glioma patients.

In order to prove the clinical impact of these laboratory findings, survival data from the EORTC / NCIC study (26981-22981/CE.3) were retrospectively analyzed for the predictive value of the MGMT promotor methylation status for the efficacy of temozolomide in addition to radiotherapy. This showed a survival benefit from chemotherapy almost exclusively for the hypermethylated glioblastoma subpopulation (Hegi *et al.*, 2005). The same crucial role for MGMT methylation status as a predictive factor for chemosensitivity in anaplastic gliomas was demonstrated in the German NOA-04 study, where newly diagnosed patients were randomized between chemotherapy and radiotherapy. MGMT promoter hypermethylation was correlated with chromosome 1p/19q deletion and predicted an improved survival after radiotherapy (Wick *et al.*, 2008). In a retrospective study in patients treated with temozolomide at first recurrence after surgery and irradiation for glioblastoma or non-1p/19q deleted anaplastic (oligo) astrocytoma, the MGMT promoter methylation was also correlated with a higher sensitivity to temozolomide (Sadones *et al.*, 2008). This study confirmed the MGMT methylation status as a valid predictor for improved time to progression (TTP) and overall survival (OS) starting from the initiation of temozolomide, in patients with anaplastic (oligo)astrocytoma, but not in glioblastoma.

Most recently, point mutations within the isocytrate dehydrogenase-1 and -2 genes have been found in gliomas (Parsons *et al.*, 2008). These mutations are found most frequently in low-grade and anaplastic glioma while they are rare in de novo glioblastoma (Ichimura *et al.*, 2009; Yan *et al.*, 2009). The presence of IDH-1 mutations is a strong positive prognostic factor for survival in all WHO-grades and also strongly correlated with the presence of chromosome 1p and 19q loss and MGMT-promoter methylation (Sanson *et al.*, 2009). Further prospective analysis in large prospective series of patients will be needed to define the exact predictive power of each of these three molecular genetic characteristics with respect to glioma chemosensitivity.

MGMT acts with a suicide mechanism, which means loss of the MGMT repair protein after having exerted its function. Thus, a prolonged exposure to temozolomide or other MGMT-consuming molecules (such as nitrosureas or O6-benzylguanine), can deplete the cellular MGMT reserves and prohibit DNA repair function (Hegi *et al.*, 2008). This significantly increases the cytotoxic effects of these

molecules. The capacity to provoke a depletion of the MGMT repair activity has already been demonstrated in mono-nuclear white blood cells. (Tolcher *et al.*, 2003) This observation has led to the hypothesis that alternative application schedules could optimize the activity of temozolomide in the treatment of glioma. Therefore, schedules with temozolomide in daily administration for 7 days or longer were developed. The most popular regimes use temozolomide for 7 days every 14 days (150 mg/m^2/day), or for 21 days every 28 days (100 mg/m^2/day) for 6 weeks every 8 weeks (75 mg/m^2/day) or daily without interruption (50 mg/m^2/day) (Brock *et al.*, 1998; Tolcher *et al.*, 2003; Wick *et al.*, 2007). In these schedules a significantly higher cumulative dose (up to 2.1 times more) is given, during the same time range of 28 days, seemingly without increasing toxicity. The only form of toxicity that is specifically associated with these alternative regimens (through diverse phase II trials), is a high incidence of lymphopenia (50-100% of patients develop a grade 3 or 4 lymphopenia) and possible increased risk of opportunistic infections. This risk might be stronger with those regimens that administer temozolomide for periods of more than 7 consecutive days without providing sufficient interruption to allow for full recovery of MGMT-levels and toxicity to the lymphocyte compartment. Following depletion, MGMT-levels need approximately 7 days to recover and levels may overshoot baseline levels by approximately 130% after 10 days of temozolomide withdrawal (Neyns *et al.*, 2008; Tolcher *et al.*, 2003). The 7 days every 14 days (150 mg/m^2/day) regimen may therefore be associated with a lesser immunosuppressive effect as compared to the regimens with more protracted administration (Wick *et al.*, 2007; Wick *et al.*, 2005). When temozolomide is administered on day 1 to 5 every week, a permanent depletion of MGMT can be expected and a better anti-tumor efficacy together with easier dose adaptation aimed for. Activity with this regimen has been reported in patients who are refractory to the 5 out of 28 day regimen (Strik *et al.*, 2008b).

In line with the preliminary case series, several phase II studies for patients with recurrent high-grade glioma showed a promising activity of some 7/14d and 21/28d schedules (Brandes *et al.*, 2006; Neyns *et al.*, 2008; Wick *et al.*, 2007). A randomized phase III trial conducted in the United Kingdom (MRC BR12) compared PCV with temozolomide (administered either by the 5 out of 28 day regimen or the 21 out of 28 day regimen according to a second randomization) as therapy at first recurrence for chemonaive patients with high-grade glioma. No statistically significant differences in survival were observed (although a trend for inferior survival with the dose dense regimen was apparent) (Lee *et al.*, 2008). In a randomized phase II trial on patients with newly diagnosed glioblastoma, however, adjuvant treatment with dose-dense temozolomide (150 mg/m^2 days 1 to 7 every 14 days) resulted in a seemingly better outcome as compared to "metronomic" (50 mg/m^2 per day continuously) temozolomide (the 1-year survival rate was 80% for the dose-dense arm and 69% for the metronomic arm) (Clarke *et al.*, 2009). An adequately powered randomized phase III trial (RTOG 0525) will compare the standard regime (RT/TMZ followed by 6 cycles 5/28d TMZ), with an experimental arm in which a 21/28d TMZ schedule is administered during the adjuvant phase. Patient recruitment for this trial was completed in 2008. This study will also attempt to prospectively determine the predictive value of the MGMT promoter methylation status for the benefit from adding temozolomide both for the standard arm and in the 21/28 regimen which is hoped to be more effective through MGMT depletion. The results of this study are expected by the second half of 2010.

Cytotoxic therapy beyond temozolomide failure

At present temozolomide is the standard of care for the initial treatment of patients with newly diagnosed glioblastoma and at first recurrence for patients with low-grade or anaplastic glioma which have been treated initially with surgery and radiation therapy. All patients, however, will experience progression of disease, many of which are in a sufficiently good state of health to receive second or third line systemic therapy. No evidence has been obtained today that any second line agent can improve the survival of patients that have failed temozolomide. Therefore such patients are best offered further treatment within the context of a prospective clinical trial. Although no data are available from any prospective clinical trial on this subject, patients who have a recurrence-free survival of more than 4 to 6 months following the end of 6 cycles of adjuvant temozolomide treatment for newly diagnosed glioblastoma may benefit from reinitiating temozolomide at recurrence (case illustration figure 1). Even at recurrence during standard temozolomide treatment, rechallenge with an alternative, dose dense schedule of temozolomide may be efficient (Strik *et al.*, 2008a). Activity of the PCV-regimen has been reported following the failure of temozolomide in patients with recurrent oligodendroglial gliomas (BOR 53%) (Van Den Bent *et al.*, 1998). Monotherapy with either CCNU or BCNU has also been considered as the legitimate control arm for randomized

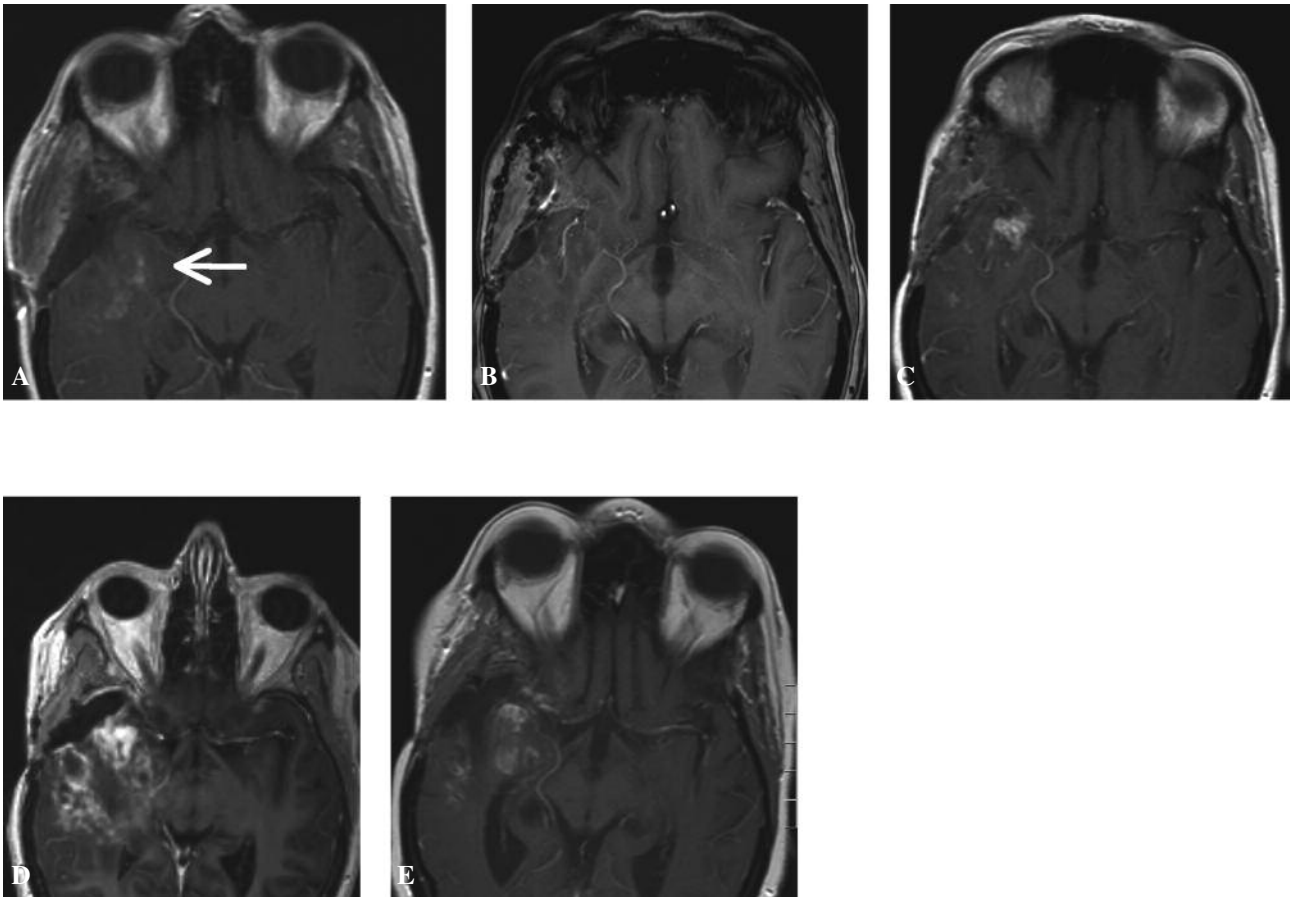

FIG. 2. — Tumor regression following first line temozolomide and, in the further course of disease, third line CCNU treatment. Following an inaugural epileptic seizure, this female patient was diagnosed at the age of 34 year with a right sided temporo-frontal non-gadolinium enhancing tumor mass. A complete resection was performed. An anaplastic oligo-astrocytoma without loss of chromosomal arms 1p or 19q was diagnosed. Postoperative radiation therapy was administered at a dose of 60 Gy. In February 2007, 4.5 years following the initial diagnosis, tumor recurrence was treated by surgery. Resection was complete except for infiltrating temporal extension (partial resection, (A, axial plane Gd enhanced T1 MRI imaging)) and followed by temozolomide chemotherapy (5 out of 28 days at a dose of 200 mg/m^2/day). A complete radiologic response was obtained after 3 cycles of temozolomide (B) and she remained progression-free for 6 months. At progression (C), the patient was subsequently treated in a phase II protocol but did not benefit from the experimental treatment (D, axial and sagittal plane images from Gd enhanced T1 MRI imaging). As third-line therapy for recurrent disease she received lomustine (CCNU) at a dose of 110 mg/m^2, once every 6 weeks, and responded to this therapy (E) for 4 months.

phase II trials that evaluate new agents in temozolomide refractory glioblastoma patients. The activity of second line nitrosurea in this setting, however, is limited, as illustrated for instance by a recently published phase II trial from the EORTC (Van Den Bent *et al.*, 2009).

Future perspectives

Currently, the EORTC is performing three phase III studies, assessing the role of temozolomide in the treatment of newly diagnosed patients with WHO grade 2 (EORTC protocol 22033 - 26033) or WHO grade 3 glioma without 1p/19q co-deletion (CATNON study; EORTC protocol 26053 - 22054); and also the role of combining radiation therapy with temozolomide in elderly patients (EORTC protocol 26062 – 22061). The low-grade glioma protocol will compare primary chemotherapy with temozolomide (75 mg/m^2/day for 21 days every 28 days) with primary radiotherapy in patients with newly diagnosed WHO grade 2 glioma. In the CATNON study (an intergroup phase III study), patients with newly diagnosed anaplastic glioma without 1p/19q chromosomal deletion are randomized between four study arms: radiation therapy alone, radiation therapy with daily concomitant, but without adjuvant temozolomide, radiation therapy with daily concomitant temozolomide followed by six adjuvant cycles of 5 out of 28 days temozolomide, and

radiation therapy without concomitant, but with 6 cycles adjuvant 5 out of 28 days temozolomide. A complementary intergroup study investigating the role of temozolomide as primary treatment for patients with a chromosomal 1p/19q co-deleted anaplastic oligodendroglioma, is currently in preparation. The protocol for elderly patients with newly diagnosed glioblastoma will randomize patients between a short course of radiation therapy with or without daily temozolomide.

Molecular targeted therapy has become a new paradigm in the treatment of cancer. The targeted agents used for the treatment of glioma have been the subject of a number of recent review articles (Djedid *et al.*, 2009; Hegi *et al.*, 2006; Idbaih *et al.*, 2008). The first receptor tyrosine kinase targeted small molecule to be widely investigated in glioma patients is imatinib, a small molecule inhibitor of c-Kit and PDGFR. Although this targeted agent has only limited activity as a single agent, preliminary results achieved with imatinib plus hydroxyurea created initial enthusiasm (Desjardins *et al.*, 2007; Raymond *et al.*, 2008). A randomized phase III trial, however, comparing hydroxyurea plus imatinib with hydroxyurea monotherapy, could not demonstrate superiority for the combination (Norden *et al.*, 2009).

The most promising results in the medical treatment of glioma are obtained with new therapeutic agents that belong to the class of angiogenesis inhibitors. High-grade gliomas are characterized by prominent formation of new blood vessels (neoangiogenesis). The Vascular Endothelial Growth Factor (VEGF) and the family of transmembrane VEGF-receptors constitute a pivotal molecular switch in this process. A considerable number of anti-angiogenic agents that target VEGF(R) are under clinical evaluation and have demonstrated activity against recurrent glioma (Batchelor *et al.*, 2007; Chamberlain, 2008; Chamberlain *et al.*, 2009a, b; Chamberlain *et al.*, 2009c; Chamberlain *et al.*, 2009d; Friedman *et al.*, 2009; Kreisl *et al.*, 2009; Vredenburgh *et al.*, 2007). Among these VEGF(R)-targeted agents are therapeutic agents that neutralize VEGF-ligand such as the monoclonal antibody bevacizumab (an IgG1 VEGF targeted mAb) or aflibercept (a protein composed of the segments of the extracellular domains of human VEGFR1 and -2) fused to the Fc-fragment of human IgG1) and small molecule receptor tyrosine kinase inhibitors (eg. Cediranib, vatalanib (PTK787/ZK2225484) (Chamberlain and Raizer, 2009d; De Groot *et al.*, 2008). Both bevacizumab and cediranib are currently most advanced in their clinical development for the treatment of high-grade glioma.

A particular challenge is the evaluation of the anti-tumor response to angiogenesis inhibitors. These new agents are very active in normalizing the tumor vasculature and the disrupted blood-brain barrier. By such, the permeability for conventional contrast media like gadolinium is reduced even if the tumor size is unchanged. Conventional MRI based patterns such as the Macdonald criteria might therefore not be as indicative for the anti-tumor effect as they are with cytotoxic chemotherapies. Extrapolation of early data on (seemingly or true) tumor response assessed by conventional response criteria based on contrast-enhanced T1 MRI (and even T2 images) on the effect on overall survival should therefore be done with caution (Norden *et al.*, 2009). At least in some patients disease progression during anti-VEGF(R) therapy will occur by diffuse non enhancing infiltration of the brain (Zuniga *et al.*, 2009). The term "pseudo-response" has been introduced very recently into the discussion on response assessment in order to address this problem. But even considering this confounding effect, a true improvement of the clinical neurological status can be achieved quite frequently and an encouraging progression-free survival is observed in a number of patients with recurrent gliomas which makes it likely that these compounds will become a valuable addition to the therapeutic repertoire (Chamberlain and Raizer, 2009d).

A first-line phase III study in which bevacizumab will be added to the standard of care with concomitant radio-chemotherapy with temozolomide followed by adjuvant temozolomide will be initiated in 2009 (AVAGLIO protocol). The multi-targeted receptor tyrosine kinase inhibitor cediranib is currently under evaluation in a phase III trial (REGAL protocol) as monotherapy and in combination with lomustine for the treatment of recurrent glioblastoma following initial RT/TMZ and adjuvant TMZ treatment. Recruitment for this study has been completed in September 2009 and the results, expected in 2010, will be the first from a randomized phase III trial to evaluate an anti-VEGFR targeted agent in the treatment of recurrent glioblastoma.

Cilengitide is another agent with anti-angiogenic activity that is currently being evaluated in a phase III study. This drug is an inhibitor of the $\alpha V\,3$ and $\alpha V\,5$ integrin receptors. In a phase II trial adding cilengitide to the standard of care with RT/TMZ followed by adjuvant TMZ, an improved outcome (as compared to an historical control) was found for patients with MGMT hypermethylated glioblastoma (Stupp *et al.*, 2007). A phase III protocol (CENTRIC protocol) is currently ongoing and will compare the current standard of care with and without cilengitide.

Only patients with methylated MGMT-promoter in the glioblastoma are eligible for study entry.

Despite the less promising results of EGFR inhibitors as a single agent in patients with recurrent glioma, this class of anti-tumoral drugs remains a possible therapeutic target for glioma treatment (Belda-Iniesta *et al.*, 2006; Combs *et al.*, 2008; Franceschi *et al.*, 2007; Neyns *et al.*, 2009). A phase II study of the EGFR tyrosine kinase inhibitor erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma achieved promising results (Prados *et al.*, 2009). A randomized phase II study (CeCil protocol), to be conducted in parallel with the CENTRIC phase III study, will recruit glioblastoma patients without MGMT promoter hypermethylation and add Cilengitide or Cetuximab to a common backbone of radiation and temozolomide therapy.

Other receptor inhibitors, targeting for instance c-MET (e.g. XL-184) or the Src family of tyrosine kinases (Dasatinib), are also in an early stage of clinical development for glioma patients and may also offer the possibility to be combined with cytotoxic agents. However, some of these new agents, e.g. temsirolimus, will have overlapping toxicities that exclude the safe combination with cytotoxic therapy (Chang *et al.*, 2005; Galanis *et al.*, 2005).

Conclusion

Temozolomide, concomitant with radiotherapy and followed by six adjuvant cycles of chemotherapy, has become the standard first-line treatment of patients with newly diagnosed glioblastoma. Temozolomide is also an established treatment option for the second-line treatment of patients with anaplastic or low-grade gliomas after failure of surgery and irradiation. The possible role of temozolomide in the primary treatment of patients with anaplastic or low-grade gliomas is currently investigated in phase III protocols. Molecular markers (MGMT promoter methylation status and chromosomal deletion of 1p and 19q) have been correlated with chemosensitivity of glioma. Their predictive value is currently validated in ongoing studies and these markers might become useful in clinical decision-making in the future. The use of second-line chemotherapy, after the failure of temozolomide, is not clearly defined despite reports on some efficacy in patients with the most chemosensitive gliomas. The introduction of new active non-chemotherapeutic drugs, especially the angiogenesis inhibitors and receptor tyrosine kinase inhibitors, is generating new hope and enthusiasm. These new agents can be combined and are being developed in combination with cytotoxic agents.

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