

Combined Modality Treatment of Glioblastoma Multiforme: The Role of Temozolomide

Carsten Nieder*, Markus Adam and Anca L. Grosu

Department of Radiation Oncology, Klinikum Rechts der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany

Abstract: Despite of improvements in the biological and molecular characterization of glioblastoma multiforme and studies of factors associated with tumor growth and progression, this type of malignant astroglial brain tumor is still difficult to treat. The present article reviews established and emerging prognostic and predictive factors and their potential influence on future therapeutic efforts. Recent developments in standard treatment options (surgery, radiotherapy and chemotherapy) are summarized. The integration of the oral cytotoxic agent temozolomide into current treatment protocols of postoperative combination therapy with radiation and drugs is discussed, especially in the context of the recently published randomized trial of the EORTC/NCIC, which showed that radiotherapy plus concomitant and adjuvant temozolomide significantly improved progression-free and overall survival over radiotherapy alone. The study also provided hypotheses about the subgroups, which are most likely to benefit from this reasonably well tolerated regimen. In a subset of patients, investigation of *MGMT* promoter methylation in tumor tissue was performed. Survival was shorter in patients with unmethylated promoter in both study groups. Patients with methylated promoter treated with radiotherapy had a median survival of 15 months, those treated with radiation plus temozolomide of 22 months ($p=0.007$). In the unmethylated group, the difference in median survival was only 1 month ($p=0.06$). Especially for these patients, alternative treatments need to be developed. The optimum schedule of temozolomide administration and the influence of combinations with additional antineoplastic agents remains to be studied. Early results of clinical trials addressing these issues are presented.

Keywords: Brain tumors, glioma, treatment, radiotherapy, chemotherapy, temozolomide.

INTRODUCTION

Primary brain tumors are a heterogeneous group of diseases arising from different cells of origin and showing characteristic age distributions [1]. Virtually all of these tumors represent less than 1% of all cancers in most western countries. Astrocytoma, one of the most common types, can be classified as low-grade or high-grade tumors. Whereas the prognosis of low-grade pilocytic astrocytoma is favorable after surgical resection alone, most World Health Organization (WHO) grade II astrocytoma will eventually fail and require radiotherapy. Nevertheless, further local progression or high-grade transformation is common. The most malignant type, glioblastoma multiforme (GBM), or WHO grade IV glioma, tends to occur in 50-70 year old patients, while the less malignant forms develop at least a decade earlier. Median survival time is limited to approximately 10-15 months for GBM and up to 30-50 months for anaplastic astrocytoma (AA) or WHO grade III astrocytoma, despite of maximal surgical resection and postoperative radiotherapy [2]. AA is histologically characterized by its increased cellularity and mitotic activity, whereas GBM shows additional necrosis or endothelial proliferation. Survival after relapse and second-line

treatment of high-grade astrocytoma is usually in the range of 6-8 months while median time to further progression was 14 weeks in over 1.400 patients treated with different regimens [3].

In diffusely infiltrating high-grade glioma, the role of combined chemo- and radiotherapy has long been unclear. Although these tumors are not curable by chemotherapy alone, rationales for combined treatment exist. Chemotherapy with different sequentially or simultaneously administered agents can be used to enhance the effect of radiotherapy, aiming either at additive cell kill or true radiosensitization, or to treat microscopic out-of-field tumor based on the principle of spatial cooperation. The main prerequisites of successful chemotherapy are sensitivity of the tumor cells to the mechanisms of the drug and sufficient drug exposure. The key issues of tumor heterogeneity with primary and acquired resistance as well as pharmacokinetics, pharmacodynamics and tumor microenvironment deserve particular attention because of several facts that are specific for brain tumors. First of all, the intact blood-brain barrier (BBB) prevents access to the brain for several compounds. Even in areas of BBB disturbance, as present for example in high-grade glioma, the effects of contemporary drug treatment are not satisfactory. Thus, achieving therapeutic concentrations in distal, seemingly intact areas that also are known to contain infiltrating tumor cells remains an enormous challenge. Various strategies of modified application or increased dose have been explored, including intraarterial, intrathecal and intratumoral delivery as well as disruption of the BBB.

*Address correspondence to this author at the Department of Radiation Oncology, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany; Tel: +49 89 4140 4501; Fax: + 49 89 4140 4880; E-mail: cnied@hotmail.com

Furthermore, many patients with brain tumors are able to metabolize chemotherapy drugs more rapidly than other tumor patients because of concomitant enzyme-inducing medications that are necessary to treat or prevent seizures. Phenytoin, carbamazepine and phenobarbital induce hepatic cytochrome P450 enzymes, resulting for example in higher maximum tolerated drug doses. It is also important to notice the possibility of decreased drug effectiveness from corticosteroid treatment [4]. Radiological assessment of the effectiveness of chemotherapy is difficult, especially in glioma patients with intense pretreatment [5]. Many groups combine radiological with clinical findings, as published by McDonald *et al.* 1990 [6]. In summary, brain tumors, especially those with high-grade histological features, present unique therapeutic challenges because of their location, aggressive biological behavior, and diffuse, infiltrative growth. Both the tumor and its treatment often result in profound changes in quality of life. Failure of local treatment is still a common feature in high-grade glioma. Thus, improvement of long-term survival rates likely requires substantial refinements of combined-modality therapy.

Prognostic Factors

Prognosis is determined by patient-associated factors (age, performance status, neurologic function, symptom indices and duration), tumor location and grade, and treatment-related factors such as surgical resectability or residual tumor volume [7]. Curran *et al.* analyzed the survival of more than 1,500 patients with high-grade glioma in the Radiation Therapy Oncology Group (RTOG) database and found that five variables (duration of symptoms, mental status, age at diagnosis, tumor grade, and postoperative performance status) defined six patient subgroups with distinct prognoses (median overall survival (OS) from 5 to 59 months) [8]. Proliferative activity measured, e.g., by variants of the Ki-67 antibody was found to correlate to WHO grade, but not to OS in multivariate analysis adjusted for the clinically established prognostic variables [9]. The same holds true for p53 immunostaining or presence of p53 gene mutations [10]. Expression of p27^{Kip1} (a cell cycle regulator), alterations in the *MMAC/PTEN* gene and epidermal growth factor receptor (EGFR) amplification in GBM are not standard assessments yet, but under active consideration by several groups. Given the complexity of assessment of a large set of potential prognosticators, it will be interesting to determine whether gene array technology will result in clinically implementable prognostic information. Recent data suggest that class prediction models, based on defined molecular profiles, classify diagnostically challenging glioma in a manner that better correlates with clinical outcome than does standard pathology [11, 12]. It might therefore be expected that better prognostic models will be available in the future.

Surgical Resection and Postoperative Radiotherapy

Surgical resection remains the initial treatment of choice. Besides establishing a tissue diagnosis, resection might lead to rapid improvement of symptoms, e.g. from mass effects, hydrocephalus etc., and reduction of steroid doses. Despite the inability to cure high-grade glioma by surgery, the

macroscopic completeness of a "T1 resection" (referring to the removal of all MR-visible enhancing tumor) is related to survival [13]. Detailed descriptions of technical surgical improvements, e.g. use of functional imaging, neuronavigation, intraoperative mapping, microsurgery, use of fluorescent tissue markers etc. can be found in recent reviews, e.g. [14]. Medical treatment aims at counteracting peritumoral edema with corticosteroids and preventing seizures with anti-convulsants.

Historically, early recurrences after resection prompted investigators to study immediate postoperative radiotherapy [15]. It was found that local fields (tumor ± edema with safety margins) are as appropriate as whole-brain radiotherapy (WBRT) and that 60 Gy are better than lower doses [16, 17]. Today, this postoperative regimen still remains an important and effective way to increase the time to progression, although it does not lead to cure in the majority of patients. Intensification of external beam radiotherapy beyond a standard course of 60 Gy over 6 weeks has been extensively investigated. Randomised studies which achieved intensification by stereotactic radiosurgery, stereotactic fractionated radiotherapy, brachytherapy and variants of conventional external beam treatment all failed to define a new standard of care [2, 18-20]. Local and marginal disease progression continued to be the most common pattern of failure.

Neither addition of radiosensitizers or enzyme inactivators such as misonidazole, etanidazole, tirapazamine, bromodeoxyuridine, -difluoromethylornithine (DFMO), or hyperbaric oxygen have resulted in significant gains [2]. Currently, newly developed radiosensitizers, e.g. Motexafin-Gadolinium (a tumorselective redox-active porphyrin) and RSR13 (an allosteric effector of hemoglobin) are under prospective clinical investigation. For patients in unfavorable prognostic groups, hypofractionated treatment to doses of 30-45 Gy over 2-3 weeks is a reasonable alternative to standard conventional radiotherapy due to increased patient convenience and better cost-effectiveness.

Postoperative Chemotherapy

Many cytotoxic drugs, most often nitrosoureas and other alkylating agents, have been added to surgery and radiotherapy since the 1970's. They were usually administered after completion of local treatment. A metaanalysis of 16 randomized clinical trials from a 17-year period suggested a moderate increase of survival of 8.6% at 2 years by adding systemic chemotherapy. Median survival increased from 9.4 to 12 months [21]. This findings was corroborated by a second metaanalysis of 3,004 patients from 12 randomized controlled trials also suggesting a small but statistically significant improvement of survival from chemotherapy [22]. Preradiation chemotherapy has resulted in disappointing results if given to adult patients with AA [23] or GBM [24, 25], possibly with the exception of temozolomide plus cisplatin [26]. In the latter trial, 45% objective responses according to McDonald's criteria [6] were noted prior to radiotherapy. Currently, a trend can be seen towards concomitant application of chemo- and radiotherapy (references [27-29] in Table 1 and [30-37] in Table 3). Certain cytotoxic drugs were found to cause

Table 1. Overview of recent combined modality studies

Author	Study Type	n	Histology GBM	Median age, years	KPS	Resection	Total dose [Gy]	Median survival	Two-year survival	Chemo-therapy
Souhami <i>et al.</i> 2004 [8]	RTOG Phase III	203	100% 40 mm	56	Median 90	?	60 vs. 60 plus SRS	13.5 vs. 13.6 Months	19 vs. 21%	BCNU
MRC 2001 [70]	Multicenter Phase III	339 335	67%	53	Median PS 1	57%	45 or 60	9.5 vs. 10 Months	15 vs. 17%	None vs. PCV
Jeremic <i>et al.</i> 2001 [43]	Single Inst. Phase II	79	77%	57	70 in 85%	71%	60	14 Months	33%	Carboplatin plus Etoposide
Weller <i>et al.</i> 2001 [46]	Single Inst. Phase II	21	100%	56	All 70	?	59.4-60	11 Months	?	Gemcitabine
Fisher <i>et al.</i> 2002 [27]	Multicenter Phase II	87	100%	?	Median 80 or 90	71%	60	9 Months	11%	Topotecan
Langer <i>et al.</i> 2001 [28]	Multicenter Phase II	61	100%	?	Median 80 or 90	75%	60	10 Months	?	Paclitaxel
Schuck <i>et al.</i> 2002 [29]	Single Inst. Phase I/II	56	61%	51	All 60	70%	60	12 Months	5% (GBM) 32% (WHO III)	Paclitaxel

MRC: Medical Research Council; RTOG: Radiation Therapy Oncology Group; GBM: Glioblastoma multiforme; KPS: Karnofsky performance status; SRS: stereotactic radiosurgery; ?: Data can not be extracted from original publication; WHO III: astrocytoma grade III; BCNU: carmustine; PCV: procarbazine, CCNU and vincristine.

radiosensitization of glioma cells *in vitro* and *in vivo* [38, 39]. In general, the optimal drug or drug combination is still a matter of debate. The results of several studies do not convincingly demonstrate superiority of polychemotherapy vs. single-agent nitrosoureas alone. These studies include a phase III trial comparing carmustine (BCNU) plus procarbazine or BCNU plus hydroxyurea, procarbazine and teniposide to single agent BCNU [40], a trial of carboplatin/etoposide followed by BCNU [41] and a recent phase III study of BCNU vs. continuous infusion BCNU plus cisplatin [42]. Several other studies of cisplatin or carboplatin also did not report improved results [43, 44]. In a recent randomised phase III trial of ACNU plus teniposide vs. ACNU plus cytarabine no significant survival difference was observed for the complete group of patients with different types of high-grade glioma or any subpopulation [45]. Phase II studies of gemcitabine, paclitaxel and topotecan showed results comparable to older regimens [27-29, 46], Table 1. In the absence of any clear survival difference between different regimens, other considerations such as toxicity, oral application and cost of treatment might guide the choice.

Several possible strategies might increase the effectiveness of chemotherapy by administering higher doses of otherwise moderately effective drugs. Intra-arterial chemotherapy, e.g. with BCNU, was comprehensively evaluated but found to be more toxic and no more effective than intravenous administration and, thus, did not offer a therapeutic gain. The latter appears to be the case for high-dose chemotherapy regimens with autologous bone marrow or peripheral blood stem cell support too [47]. Results reported so far are less encouraging than anticipated. Treatment-related mortality was as high as 10-15%. Biodegradable polymers may be impregnated with cytotoxic

chemotherapeutic drugs, such as BCNU, and the polymer wafers placed into the tumor bed during surgery, possibly exposing tumor cells to higher drug concentrations. A randomised trial of BCNU vs. placebo wafers demonstrated a statistically significant increase in median survival (13.9 vs. 11.6 months) for the BCNU wafer-group; however, the “wafer” trials have never directly compared the active wafer against conventionally administered chemotherapy [48]. Other compounds such as bucladesine and 5-fluorouracil are also under investigation for local delivery. Convection-enhanced delivery (CED) can be used to perfuse regions of the brain with therapeutic agents in a manner that bypasses the BBB. In animal studies, encouraging results have been obtained. Clinical trials of CED are underway, also for studies of toxin-conjugates. Another way of improving the results might be the development of better cytotoxic drugs. In the 1990’s, temozolomide has become available for clinical trials, initially in recurrent glioma. After oral administration, temozolomide crosses the BBB. The compound needs conversion into its active form to methylate the O⁶ and N⁷ positions of guanine bases. It depletes the DNA-repair enzyme O⁶ methyl-guanine DNA methyl-transferase (MGMT) [49], which is involved in resistance to chemotherapy. The following paragraph summarizes the results of the major temozolomide trials.

Temozolomide for GBM

Temozolomide has been shown in GBM at first relapse to prolong PFS and to maintain neurological functioning and performance status for a longer time than procarbazine [50, 51]. Table 2 provides an overview on clinical trials for recurrent high-grade glioma. Response rates and time to progression vary with patient selection, particularly the

Table 2. Results of temozolomide chemotherapy for recurrent malignant glioma. The studies included some patients with oligodendroglioma, transformed low-grade glioma and more than one recurrence. Diagnosis of recurrence was based on imaging criteria

Reference	n	Treatment	Pre-treatment	CHT	Age	KPS	Non-GBM	MOS	TTP	CR+PR
Bower <i>et al.</i> 1997 [52]	103	Temozolomide Med. 4 cycles	Individual	30%	44	WHO I	20%	25 W.	17 W.	?
Yung <i>et al.</i> 1999 [53]	162	Temozolomide Med. 5 cycles	Individual	60%	42	80%	88%	61 W.	24 W.	35%
Chang <i>et al.</i> 2004 [54]	213	Temozolomide Med. 2 cycles	Individual	82% 51%*	42 53*	80%	33%	49 W. 32 W.*	21 W. 10 W.*	16%
Brandes <i>et al.</i> 2004 [55]	50	Temozolomide plus cisplatin	OP+RT	0%	53	80%	0%	48 W.	18 W.	20%
Silvani <i>et al.</i> 2004 [56]	33	Temozolomide plus cisplatin	OP+RT+CHT	100%	54	70%	39%	?	33 W.	19%
Prados <i>et al.</i> 2004 [57]	38	Temozolomide plus BCNU	Individual	10.5%	53	80%	0%	34 W.	11 W.	6%
Chua <i>et al.</i> 2004 [58]	22	Temozolomide plus caelyx	Individual	9%	55	ECOG I	0%	35 W.	14 W.	19%
Spence <i>et al.</i> 2004 [59]	16	Temozolomide plus tamoxifen	Individual	44%	48	60%	38%	?	10 W.	6%

OP: surgical resection; RT: radiotherapy; CHT: chemotherapy, percentage of patients with one or more previous chemotherapy regimen(s); Age: median in years (some papers reported mean rather than median value); KPS: median Karnofsky performance status or other classification (some papers reported mean values); Non-GBM: original histology at initial diagnosis other than glioblastoma multiforme; MOS: median overall survival from treatment of recurrence in weeks; TTP: median time to further progression in weeks; CR+PR: complete or partial remission based on imaging and McDonald's criteria [6]; ?: Data can not be extracted from original publication; * data for grade III tumors and GBM, respectively.

number of patients with GBM. Most studies reported a time to progression in the order of 3-5 months [52-59]. Adding other drugs to temozolomide has not resulted in improved results [56, 58, 59].

The drug has also been administered to elderly patients with newly diagnosed GBM in a phase II trial [60]. In this subgroup with unfavorable prognosis, survival usually is limited to 5-7 months [8]. Most centers have treated such patients either with short-course radiotherapy or best supportive care. Chinot *et al.* administered temozolomide 150-200 mg/m²/day for 5 consecutive days on a 28-day cycle as sole treatment until progression (32 patients, median age 75 years, median Karnofsky performance status 70%). Nine patients achieved a partial response [60]. Median PFS was 5 months and OS 6.4 months. Compared to radiotherapy, no obvious improvement can be seen. Therefore, data about quality of life and costs of treatment are needed to define the best strategies for elderly patients with GBM.

A large phase III and a smaller randomized phase II trial of different temozolomide administration schedules in addition to radiotherapy have been published [30, 31]. These trials were conducted after encouraging survival data (median 16 months for patients with newly diagnosed GBM) were observed in a non-randomized phase II study of radiotherapy with 60 Gy and both concomitant and adjuvant temozolomide [32]. The outcome of these studies is shown in Table 3. Noteworthy is a different administration schedule for adjuvant temozolomide in the phase II study by Athanassiou *et al.*, aiming at dose intensification [31]. In this study, a significant improvement in outcome compared to the

arm with radiotherapy alone was found. The median time to progression was 5.2 months after radiotherapy alone, however, it was 10.8 months after combined treatment ($p < 0.0001$). There was also a 5 months increase in overall survival. Eligibility criteria for the large phase III trial conducted by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) included: age, 18-70 years, WHO performance status of 2 or less, newly diagnosed GBM, stable or decreasing dose of corticosteroids for at least 14 days before randomisation and adequate hematologic, renal and hepatic function. Patients were stratified according to performance status, extent of surgery and treatment center. The control group received standard focal radiotherapy to 60 Gy ($n=286$). The chemotherapy group received the same radiotherapy plus both concurrent and adjuvant temozolomide for a maximum of 6 cycles ($n=287$). The primary endpoint was OS and the sample size was calculated to detect a 33% increase with a power of 80% at a significance level of 0.05. Tumor progression was defined as an increase in size by 25%, the appearance of new lesions, or an increased need for corticosteroids. The baseline characteristics of the patients were well balanced, except for corticosteroid treatment at the time of randomisation (75% in the radiotherapy arm vs. 67% in the temozolomide plus radiotherapy arm). The median time from diagnosis to start of treatment was 5 weeks in both groups. Discontinuation of temozolomide for toxicity reasons was recorded in 13% and 85% completed combined radiotherapy and temozolomide as planned. Seventy-eight percent of the patients started adjuvant temozolomide and 47% completed 6 cycles. Only 8% discontinued because of

Table 3. Overview of recent combined modality studies with temozolomide

Author	Study Type	n	Histology GBM	Median Age in Years	KPS	Resection	Total Dose [Gy]	Median Survival	Two-year Survival	Chemotherapy
Stupp <i>et al.</i> 2005 [30]	Multi-center Phase III	287 286	92% 93%	56 57	86% WHO 0/1 87% WHO 0/1	83% 84%	60 60	15 Months 12 Months	27% 10%	Temozolomide ⁷ vs. radiotherapy alone (randomized)
Athanassiou <i>et al.</i> 2005 [31]	Multi-center Phase II	57 53	100% 100%	? ?	30% >80 51% >80	58% 58%	60 60	13 Months 8 Months	16% ?	Temozolomide ¹ vs. radiotherapy alone (randomized)
Stupp <i>et al.</i> 2002 [32]	Two-center Phase II	64	100%	52	90-100 in 64%	76%	60	16 Months	31%	Temozolomide ⁶
Lanzetta <i>et al.</i> 2003 [35]	Single Inst. Phase II	21	100%	44	90-100 in 67%	85%	60	16 Months	?	Temozolomide ⁶
Kocher <i>et al.</i> 2005 [33]	Single Inst. Phase II	69	68%	52	Median 80	67% (complete)	60	15 Months (GBM)	24% (GBM)	Temozolomide ⁴
Combs <i>et al.</i> 2004 [34]	Single Inst. Phase I/II	53	100%	?	?	68%	60	19 Months	29%	Temozolomide ⁸
Butowski <i>et al.</i> 2005 [36]	Single Inst. Phase II	61	100%	54	Median 90	84%	60	13 Months	20%	Temozolomide ² plus cis-retinoic acid
Chang <i>et al.</i> 2004 [39]	Single Inst. Phase II	67	100%	51	Median 90	80%	60	17 Months	27%	Temozolomide ³ plus thalidomide
Balana <i>et al.</i> 2004 [26]	Multi-center Phase II	40	100%	58	Median 80	63%	60	12.5 Months	11%	Temozolomide ⁵ and cisplatin
Barrie <i>et al.</i> 2005 [71]	Single Inst. Phase II	40	100%	61	Median 70	0%	60	13 Months	15%	Temozolomide ⁹ and BCNU

GBM: Glioblastoma multiforme; KPS: Karnofsky performance status; ?: Data can not be extracted from original publication; ¹ 75 mg/m²/day during radiotherapy and 150 mg/m²/day on days 1 through 5 and 15 to 19 every 4 weeks for 6 cycles; ² 75 mg/m²/day during radiotherapy and 150-200 mg/m²/day for 5 days every 4 weeks for up to 1 year, depending on toxicity and local control; ³ 150-200 mg/m²/day for 5 days every 4 weeks starting on the first day of radiotherapy for up to 1 year, depending on toxicity and local control; ⁴ 75 mg/m²/day with weekend break during radiotherapy only; ⁵ 200 mg/m²/day for 5 days every 4 weeks plus cisplatin 100 mg/m² on day 1 for 3 cycles before radiotherapy; ⁶ 75 mg/m²/day during radiotherapy and 200 mg/m²/day for 5 days every 4 weeks for 6 cycles; ⁷ 75 mg/m²/day during radiotherapy and 150-200 mg/m²/day for 5 days every 4 weeks for 6 cycles; ⁸ 50 mg/m²/day during radiotherapy only; ⁹ 110 mg/m²/day for 5 days plus BCNU 150 mg/m²/day on day 1 every 42 days for up to 4 cycles, followed by radiotherapy (n=33) and then further chemotherapy cycles (n=14), 40% progressed during pre-radiation chemotherapy.

toxicity. The median number of post-radiation cycles was 3. All major side effects are summarized in Table 4. The median OS was 14.6 vs. 12.1 months (hazard ratio for death 0.63, p<0.001). A comparable significance level resulted for PFS (6.9 vs. 5.0 months). The difference between PFS and OS is surprisingly large, possibly as a result of second-line treatment and/or close follow-up with early detection of progression. No significant survival improvement was found in patients with biopsy only (n=93) and in patients with poor performance status (WHO 2, n=70) [30].

Even if comparisons between both randomized trials are difficult, a few points deserve further explanation. The fact

that OS was shorter in both groups of the randomized phase II trial can be explained by a higher number of patients with biopsy only. The median time to progression after radiotherapy alone was in good agreement (5.2 months and 5.0 months, respectively). However, it was 10.8 months after combined treatment in the randomized phase II trial, which is much longer than in the EORTC/NCIC trial (6.9 months). Survival from progression was shorter in the Athanassiou *et al.* trial (2.6 and 2.5 months) than in the EORTC/NCIC trial (7.7 vs. 7.1 months), where more patients received further salvage treatment after progression. In summary, both randomized trials found significant differences in median OS

Table 4. Toxicity and adverse events as reported by Stupp *et al.* 2005 [30], Athanassiou *et al.* 2005 [31] (in parentheses, data reported only for hematologic toxicities in the combined radiochemotherapy arm), Grossman *et al.* 2003 [42] and Weller *et al.* 2003 [45]

Toxicity	RT alone [30]	RT plus temozolomide [30] and [31]	RT plus BCNU [42]	RT plus ACNU and teniposide [45]
Hematologic grade 3 or 4	none	Concomitant phase: 7% (9%) Adjuvant phase: 14% (7%)	32%	24%
Severe infections	2%	Concomitant phase: 3% Adjuvant phase: 5%	5%	8%
Fatigue grade 2-4	30%	51%	Not reported	Not reported
Nausea/vomiting grade 2-4	4%	30%	Not reported	Not reported
Thromboembolic events	3%	2%	Not reported	4%
All grade 3 toxicities	15%	31%	65%	Not reported

RT: radiotherapy.

and PFS. However, the latter reached not more than 11 months and the survival curves suggest that few if any patients can be cured by this treatment. The best dose and treatment duration for temozolomide is yet undefined (simultaneous only or also post-radiation and if, for 6 or more cycles?). Intriguing data by Kocher *et al.* suggest that administration of temozolomide for 5 days per week during radiotherapy without any adjuvant treatment might result in comparable outcome as in the EORTC/NCIC trial (median PFS 7.3 months, OS 14.6 months) [33]. A second single-institution trial without adjuvant treatment has been reported as abstract only (Table 3) [34]. The preliminary data are encouraging (median PFS 8 months, OS 19 months). However, no head to head comparison is available. The economic impact of adjuvant temozolomide certainly justifies a randomized phase III trial. If such a trial should demonstrate equivalence of these two regimens, the next steps would probably be the search for both other radiosensitizing agents to improve simultaneous radiotherapy plus temozolomide further and effective adjuvant treatment approaches. Unfortunately, addition of further agents to this regimen so far has not resulted in additional benefit (Table 3). In the light of the increased costs of the newly established EORTC/NCIC radiotherapy plus temozolomide regimen, a large U.S. intergroup trial is currently comparing temozolomide vs. BCNU plus radiotherapy.

Resistance of tumor cells to cytotoxic drugs is a major problem. Possible resistance mechanisms include the cell membrane protein P-glycoprotein (PGP), an energy-dependent drug efflux pump removing a wide range of lipophilic chemotherapy agents. PGP expression has been described in tumor blood vessels as well as neoplastic cells of high-grade glioma [61]. Another mechanism is intracellular drug inactivation or transformation as a result of increased concentrations of detoxifying enzymes such as glutathione S-transferases (GST), MGMT or poly (ADP-ribose) polymerase (PARP). GST catalyzes the conjugation of glutathione with a large number of compounds with an electrophilic center, including chemotherapeutic agents. Nitrosoureas may be deactivated by denitrosylation *via* GST or methylation by MGMT. Belanich *et al.* showed that BCNU-treated patients with high levels of MGMT had a

significantly shorter time to progression and OS than those with lower levels [62]. Friedman *et al.* reported that MGMT-level might be a valuable predictive factor for response to temozolomide [63]. It has recently been investigated whether MGMT promoter methylation in GBM tissue from 206, i.e. 36% of all, patients in the randomized EORTC/NCIC trial is associated with a benefit from temozolomide [64]. Of these samples, 45% had detectable methylation, which leads to a loss of MGMT expression and reduced DNA repair capacity. Unrepaired lesions might trigger cell-death cascades. Consistent with these facts, OS was better in patients with methylated promoter in both groups (radiotherapy and radiotherapy plus temozolomide). Furthermore, patients with methylated promoter and reduced MGMT expression treated with radiotherapy had a median OS of 15 months, those treated with radiation plus temozolomide of 22 months ($p=0.007$). In the unmethylated group, the difference in median OS was only 1 month ($p=0.06$). Especially for these patients, alternative treatments need to be studied. Pretreatment with O⁶-methylguanine, which inactivates the enzyme, may overcome resistance.

Perspectives

Multimodal treatment approaches for high-grade glioma include the components of surgical resection, postoperative radiotherapy and additive chemotherapy. In certain prognostic subgroups of patients, the role of chemotherapy is still not established yet. In GBM patients with favorable prognostic factors a shift from nitrosourea-based regimens to temozolomide has started after the EORTC/NCIC trial, because temozolomide was effective, acceptably well tolerated and can be administered orally. However, several questions concerning the optimal temozolomide-based regimen and its economic impact remain unanswered. Molecular studies have identified promising new targets for therapeutic intervention, e.g. with tyrosine kinase inhibitors and antibodies, whose efficacy and safety are now being studied [65-69]. The current experience in cancer treatment shows that several targets should be approached to provide maximal chances of cure and that it is unlikely for a single therapeutic measure to be applicable to all patients. This

includes targeting the same signal transduction pathway at different levels with different compounds. Therefore, rational combinations between established treatments and new approaches, aiming for example at inhibition of angiogenesis, induction of apoptosis, or inhibition of several signal transduction pathways, might offer the best opportunity to improve the prognosis. Laboratory correlative studies of post-treatment tumor analyses will help to define both efficacy and optimal biologic dose. Nevertheless, the treatment of high-grade glioma remains challenging. Any new treatment modality must face the difficulty of balancing the desirable effects on relatively resistant tumor cells and the potential negative impact on quality of life in patients with limited life expectancy. In addition, accessing the diffusely infiltrating tumor cells within the normal brain is not a trivial task. A crucial point will be to learn how to integrate new approaches into existing treatment algorithms and to take enough time to optimize such strategies, for example with regard to dose-effect relationship. PFS at 6 months is now being used as endpoint in many clinical studies, especially in recurrent/progressive disease, because it has been shown to be a useful surrogate marker for OS. This change of endpoint facilitates rapid evaluation of new strategies. Better measures of tumor response may need to be developed in addition to standard response criteria such as radiographic response and time to progression, which do not imply improved quality of life. Such data will also be needed to provide arguments in the discussion about toxicity and economic aspects of more aggressive multimodal treatment in a disease where progress towards improved prognosis will develop slowly.

REFERENCES

- [1] Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. *Acta Neuropathol (Berl.)* 2005; 109: 93-108.
- [2] Nieder C, Andratschke N, Wiedenmann N, Busch R, Grosu AL, Molls M. Radiotherapy for high-grade gliomas: does altered fractionation improve the outcome? *Strahlenther Onkol* 2004; 180: 401-7.
- [3] Huncharek M, Muscat J. Treatment of recurrent high grade astrocytomas: results of a systematic review of 1415 patients. *Anticancer Res* 1998; 18: 1303-11.
- [4] Wolff J, Denecke J, Jurgens H, *et al.* Dexamethasone induces partial resistance to cisplatin in C6 glioma cells. *Anticancer Res* 1996; 16: 805-10.
- [5] Vos MJ, Uitdehaag BM, Barkhof BM, *et al.* Interobserver variability in the radiological assessment of response to chemotherapy in glioma. *Neurology* 2003; 60: 826-30.
- [6] McDonald DR, Cascino TL, Schold SC, *et al.* Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990; 8: 1277-80.
- [7] Laws ER, Parney IF, Huang W, *et al.* Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the Glioma Outcomes Project. *J Neurosurg* 2003; 99: 467-73.
- [8] Curran WJ, Scott CB, Horton J, *et al.* Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst* 1993; 85: 704-10.
- [9] Stemmer-Rachamimov AO, Louis DN. Histopathologic and immunohistochemical prognostic factors in malignant gliomas. *Curr Opin Oncol* 1997; 9: 230-4.
- [10] Nieder C, Petersen S, Petersen C, Thames HD. The challenge of *p53* as prognostic and predictive factor in gliomas. *Cancer Treat Rev* 2000; 26: 67-73.
- [11] Nutt CL, Mani DR, Betensky RA, *et al.* Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res* 2003; 63: 1602-7.
- [12] Shai R, Shi T, Kremen TJ, *et al.* Gene expression profiling identifies molecular subtypes of gliomas. *Oncogene* 2003; 22: 4918-23.
- [13] Keles GE, Anderson B, Berger MS. The effect of extent of resection on time to tumor progression and survival in patients with glioblastoma multiforme of the cerebral hemisphere. *Surg Neurol* 1999; 52: 371-9.
- [14] Schiff D, Shaffrey ME. Role of resection for newly diagnosed malignant gliomas. *Expert Rev Anticancer Ther* 2003; 3: 621-30.
- [15] Walker MD, Alexander E, Hunt WE, *et al.* Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. *J Neurosurg* 1978; 49: 333-43.
- [16] Walker MD, Strike TS, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys* 1979; 5: 1725-31.
- [17] Bleehen NM, Stenning SP. A Medical Research Council trial of two radiotherapy doses in the treatment of grades 3 and 4 astrocytoma. *Br J Cancer* 1991; 64: 769-74.
- [18] Laperriere NJ, Leung PM, McKenzie S, *et al.* Randomized study of brachytherapy in the initial management of patients with malignant astrocytoma. *Int J Radiat Oncol Biol Phys* 1998; 41: 1005-11.
- [19] Selker RG, Shapiro WR, Burger P, *et al.* The Brain Tumor Cooperative Group NIH Trial 87-01: a randomised comparison of surgery, external radiotherapy, and carmustine versus surgery, interstitial radiotherapy boost, external radiation therapy, and carmustine. *Neurosurgery* 2002; 51: 343-53.
- [20] Souhami L, Seiferheld W, Brachman D, *et al.* Randomized comparison of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme: report of Radiation Therapy Oncology Group 93-05 protocol. *Int J Radiat Oncol Biol Phys* 2004; 60: 853-60.
- [21] Fine HA, Dear KB, Loeffler JS, *et al.* Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. *Cancer* 1993; 71: 2585-97.
- [22] Stewart LA. Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials. *Lancet* 2002; 359: 1011-8.
- [23] Rao RD, Krishnan S, Fitch TR, *et al.* Phase II trial of carmustine, cisplatin, and oral etoposide chemotherapy before radiotherapy for grade 3 astrocytoma (anaplastic astrocytoma): results of North Central Cancer Treatment Group trial 98-72-51. *Int J Radiat Oncol Biol Phys* 2005; 61: 380-6.
- [24] Raymond E, Fabbro M, Boige V, *et al.* Multicentre phase II study and pharmacokinetic analysis of irinotecan in chemotherapy-naive patients with glioblastoma. *Ann Oncol* 2003; 14: 603-14.
- [25] Mane JM, Fernandez R, Munoz A, *et al.* Preradiation chemotherapy with VM-26 and CCNU in patients with glioblastoma multiforme. *Tumori* 2004; 90: 562-6.
- [26] Balana C, Lopez-Pousa A, Berrocal A, *et al.* Phase II study of temozolomide and cisplatin as primary treatment prior to radiotherapy in newly diagnosed glioblastoma multiforme patients with measurable disease. *J Neurooncol* 2004; 70: 359-69.
- [27] Fisher B, Won M, McDonald D, *et al.* Phase II study of topotecan plus cranial radiation for glioblastoma multiforme: results of Radiation Therapy Oncology Group 9513. *Int J Radiat Oncol Biol Phys* 2002; 53: 980-6.
- [28] Langer CJ, Ruffer J, Rhodes H, *et al.* Phase II Radiation Therapy Oncology Group trial of weekly paclitaxel and conventional external beam radiation therapy for supratentorial glioblastoma multiforme. *Int J Radiat Oncol Biol Phys* 2001; 51: 113-9.
- [29] Schuck A, Muller SB, Kohler A, *et al.* Combined radiochemotherapy with paclitaxel in the treatment of malignant glioma. *Strahlenther Onkol* 2002; 178: 486-90.
- [30] Stupp R, Mason WP, van den Bent MJ, *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; 352: 987-96.
- [31] Athanassiou H, Synodinou M, Maragoudakis E, *et al.* Randomized phase II study of temozolomide and radiotherapy with radiotherapy

- alone in newly diagnosed glioblastoma multiforme. *J Clin Oncol* 2005; 23: 2372-7.
- [32] Stupp R, Dietrich PY, Ostermann Kraljevic S, *et al.* (2002) Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. *J Clin Oncol* 20:1375-1382
- [33] Kocher M, Kunze S, Eich HT, Semrau R, Muller RP. Efficacy and toxicity of postoperative temozolomide radiochemotherapy in malignant glioma. *Strahlenther Onkol* 2005; 181: 157-63.
- [34] Combs SE, Gutwein S, Schulz-Ertner D, *et al.* Phase I/II study of temozolomide combined with radiation as postoperative treatment in primary glioblastoma multiforme (Abstract). *Int J Radiat Oncol Biol Phys* 2004; 60 Suppl: S258-9.
- [35] Lanzetta G, Campanella C, Rozzi A, *et al.* Temozolomide in radiochemotherapy combined treatment for newly-diagnosed glioblastoma multiforme: phase II clinical trial. *Anticancer Res* 2003; 23: 5159-64.
- [36] Butowski N, Prados MD, Lamborn KR, *et al.* A phase II study of concurrent temozolomide and cis-retinoic acid with radiation for adult patients with newly diagnosed supratentorial glioblastoma. *Int J Radiat Oncol Biol Phys* 2005; 61: 1454-9.
- [37] Wedge SR, Porteous JK, Glaser MG, *et al.* In vitro evaluation of temozolomide combined with X-irradiation. *Anticancer Drugs* 1997; 8: 92-7.
- [38] Van Rijn J, Heimans JJ, van den Berg J, *et al.* Survival of human glioma cells treated with various combinations of temozolomide and X-rays. *Int J Radiat Oncol Biol Phys* 2000; 47: 779-84.
- [39] Chang SM, Lamborn KR, Malec M, *et al.* Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme. *Int J Radiat Oncol Biol Phys* 2004; 60: 353-357.
- [40] Shapiro WR, Green SB, Burger PC, *et al.* Randomized trial of three chemotherapy regimens and two radiotherapy regimens in postoperative treatment of malignant glioma. *Brain Tumor Cooperative Group Trial 8001. J Neurosurg* 1989; 71: 1-9.
- [41] Brandes AA, Rigon A, Zampieri P, *et al.* Carboplatin and teniposide concurrent with radiotherapy in patients with glioblastoma multiforme: a phase II study. *Cancer* 1998; 82: 355-61.
- [42] Grossman SA, O'Neill A, Grunnet M, *et al.* Phase III study comparing three cycles of infusional carmustine and cisplatin followed by radiation therapy with radiation therapy and concurrent carmustine in patients with newly diagnosed supratentorial glioblastoma multiforme: Eastern Cooperative Oncology Group Trial 2394. *J Clin Oncol* 2003; 21: 1485-91.
- [43] Jeremic B, Shibamoto Y, Grujicic D, *et al.* Concurrent accelerated hyperfractionated radiation therapy and carboplatin/etoposide in patients with malignant glioma: long-term results of a phase II study. *J Neurooncol* 2001; 51: 133-41.
- [44] Levin VA, Yung WK, Bruner J, *et al.* Phase II study of accelerated fractionation radiation therapy with carboplatin followed by PCV chemotherapy for the treatment of anaplastic gliomas. *Int J Radiat Oncol Biol Phys* 2002; 53: 58-66.
- [45] Weller M, Muller B, Koch R, *et al.* Neuro-Oncology Working Group 01 trial of nimustine plus teniposide versus nimustine plus cytarabine chemotherapy in addition to involved-field radiotherapy in the first-line treatment of malignant glioma. *J Clin Oncol* 2003; 21: 3276-84.
- [46] Weller M, Streffer J, Wick W, *et al.* Preirradiation gemcitabine chemotherapy for newly diagnosed glioblastoma. *Cancer* 2001; 91: 423-7.
- [47] Durando X, Lemaire JJ, Tortochaux J, *et al.* High-dose BCNU followed by autologous hematopoietic stem cell transplantation in supratentorial high-grade malignant gliomas: a retrospective analysis of 114 patients. *Bone Marrow Transplant* 2003; 31: 559-64.
- [48] Westphal M, Hilt DC, Bortey E, *et al.* A phase III trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-oncol* 2003; 5: 79-88.
- [49] Tolcher AW, Gerson SL, Denis L, *et al.* Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br J Cancer* 2003; 88: 1004-11.
- [50] Osoba D, Brada M, Yung WK, *et al.* Health-related quality of life in patients treated with Temozolomide versus Procarbazine for recurrent glioblastoma multiforme. *J Clin Oncol* 2000; 18: 1481-91.
- [51] McDonald DR, Kiebert G, Prados M, *et al.* Benefit of temozolomide compared to procarbazine in treatment of glioblastoma multiforme at first relapse: effect on neurological functioning, performance status and health related quality of life. *Cancer Invest* 2005; 23: 138-44.
- [52] Bower M, Newlands ES, Bleeche NM, *et al.* Multicentre CRC phase II trial of temozolomide in recurrent or progressive high-grade glioma. *Cancer Chemother Pharmacol* 1997; 40: 484-8.
- [53] Yung WKA, Prados MD, Yaya-Tur R, *et al.* Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. *J Clin Oncol* 1999; 17: 2762-71.
- [54] Chang SM, Theodosopoulos P, Lamborn K, *et al.* Temozolomide in the treatment of recurrent malignant glioma. *Cancer* 2004; 100: 605-11.
- [55] Brandes AA, Basso U, Reni M, *et al.* First-line chemotherapy with cisplatin plus fractionated temozolomide in recurrent glioblastoma multiforme: a phase II study of the Gruppo Italiano Cooperativo di Neuro-Oncologia. *J Clin Oncol* 2004; 22: 1598-1604
- [56] Silvani A, Eoli M, Salmaggi A, *et al.* Phase II trial of cisplatin plus temozolomide, in recurrent and progressive glioma patients. *J Neurooncol* 2004; 66: 203-8.
- [57] Prados MD, Yung WK, Fine HA, *et al.* Phase 2 study of BCNU and temozolomide for recurrent glioblastoma multiforme: North American Brain Tumor Consortium study. *Neuro-oncol* 2004; 6: 33-7.
- [58] Chua SL, Rosenthal MA, Wong SS, *et al.* Phase 2 study of temozolomide and Caelyx in patients with recurrent glioblastoma multiforme. *Neuro-oncol* 2004; 6: 38-43.
- [59] Spence AM, Peterson RA, Scharnhorst JD, Silbergeld DL, Rostomily RC. Phase II study of concurrent continuous Temozolomide and Tamoxifen for recurrent malignant astrocytic gliomas. *J Neurooncol* 2004; 70: 91-5.
- [60] Chinot OL, Barrie M, Frauger E, *et al.* Phase II study of temozolomide without radiotherapy in newly diagnosed glioblastoma multiforme in elderly populations. *Cancer* 2004; 100: 2208-14.
- [61] Von Bossanyi P, Diete S, Dietzmann K, *et al.* Immunohistochemical expression of P-glycoprotein and glutathione S-transferases in cerebral gliomas and response to chemotherapy. *Acta Neuropathol* 1997; 94: 605-11.
- [62] Belanich M, Pastor M, Randall T, *et al.* Retrospective study of the correlation between DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. *Cancer Res* 1996; 56: 783-8.
- [63] Friedman HS, McLendon RE, Kerby T, *et al.* DNA mismatch repair and O6-alkylguanine-DNA alkyltransferase analysis and response to temodal in newly diagnosed malignant glioma. *J Clin Oncol* 1998; 16: 3851-7.
- [64] Hegi ME, Diserens AC, Gorlia T, *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; 352: 997-1003.
- [65] Chakravarti A, Chakladar A, Delaney MA, *et al.* The epidermal receptor growth factor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. *Cancer Res* 2002; 62: 4307-15.
- [66] Eshleman JS, Carlson BL, Mladek AC, *et al.* Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation therapy. *Cancer Res* 2002; 62: 7291-7.
- [67] Choe G, Horvath S, Cloughesy TF, *et al.* Analysis of the phosphatidylinositol 3'-kinase signalling pathway in glioblastoma patients in vivo. *Cancer Res* 2003; 63: 2742-6.

- [68] Lammering G, Hewit TH, Valerie K, *et al.* Anti-erbB receptor strategy as a gene therapeutic intervention to improve radiotherapy in malignant human tumours. *Int J Radiat Biol* 2003; 79: 561-8.
- [69] Nieder C, Schlegel J, Andratschke N, Thamm R, Grosu AL, Molls M. The role of growth factors in central nervous system tumours. *Anticancer Res* 2003; 23: 1681-6.
- [70] Medical Research Council Brain Tumour Working Party. Randomized trial of procarbazine, lomustine, and vincristine in the adjuvant treatment of high-grade astrocytoma: a Medical Research Council trial. *J Clin Oncol* 2001; 19: 509-18.
- [71] Barrie M, Couprie C, Dufour H, *et al.* Temozolomide in combination with BCNU before and after radiotherapy in patients with inoperable newly diagnosed glioblastoma multiforme. *Ann Oncol* 2005; 16: 1177-84.

Received: May 06, 2005

Revised: July 07, 2005

Accepted: July 11, 2005