A Phase II Study of Cisplatin and Temozolomide in Heavily Pre-treated Patients with Temozolomide-refractory High-grade Malignant Glioma

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Abstract. *Background: There is pre-clinical evidence of synergism between cisplatin and temozolomide due to higher inhibition of O^6-alkyl-guanine-alkyltransferase (AGAT), an enzyme involved in the mismatch repair system and in the mechanisms of drug resistance to alkylating agents. Patients and Methods: Heavily pre-treated patients with temozolomide-refractory high-grade malignant glioma received cisplatin at a dose of 75 mg/m^2 on day 1 and temozolomide at a dose of 150 mg/m^2 on days 1 to 5 every 21 days until progression or major toxicity. Results: Twenty-four patients were enrolled and a total of 96 cycles were delivered (median for each patient=4). Toxicity was manageable and mostly grade 1-2: haematological, gastroenterological (nausea and vomiting) and fatigue. In patients with glioblastoma, an overall response rate of 29.4% was achivied, with no complete response, and with a disease control rate (responses plus stabilizations) of 64.7% . The median time to progression was 3.8 months (95% confidence interval 2.4-6.8), progression-free survival at 6 months was 28% and overall survival was 7.0 months (95% confidence interval 4.8-11.0). Conclusion: The combination of temozolomide and cisplatin is safe and moderately effective in the treatment of heavily pre-treated patients with relapsed high-grade glioma refractory to single-agent temozolomide.*

Malignant gliomas are relatively rare diseases. Prognosis is poor but has improved in recent years due to an improvement of the multidisciplinary approach: earlier diagnosis; better and repeated surgery plus local chemotherapy, such as carmustine wafers (1); improved radiotherapy; and more effective systemic chemotherapy, such as temozolomide (2). Long-term survivors are increasingly more frequent in our clinical practice and the median overall survival (OS) of patients with glioblastoma reached 15 months in a phase III study and about 20 months, for selected patients in phase II studies (3).

Unfortunately, there is no effective chemotherapy for patients progressing during or after temozolomide therapy. Surgery or further chemotherapy always needs to be considered whenever possible. Some data using temozolomide with modified schedules are available, the old procarbazine, CCNU and vincristine (PCV) regimen, irinotecan and carmustine, fotemustine and carmustine, among others. All these attempts at treatment are characterized by low response rates (RR) and a lack of durability when responses occur.

Before there was evidence of the great efficacy obtained with the use of the new antiangiogenetics, for instance bevacizumab in combination with irinotecan [RR 57% , 6-month progression-free survival (PFS-6) of 46% (4)], we exploited the synergism between temozolomide and cisplatin. Temozolamide acts through methylation of the O^6 position of guanylic acid in DNA with intrachain links. Consequent mismatch repair system activation leads to apoptotic cell death. Resistance to temozolomide is due to high intracellular levels of O^6-alkyl-guanine-alkyltransferase (AGAT) which is involved in DNA-repair processes transferring an alkyl group from DNA to another cysteine residue. Therefore, cells with increased concentrations of AGAT, or with a deficiency in mismatch repair before drug administration, may be resistant to temozolomide (5). D'Atri *et al.* (6) showed *in vitro* how cisplatin is able to reduce intracellular AGAT levels in proportion to the drug concentration and to the duration of cell exposure, with a nadir after 24-48 hours. The effect

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Key Words: Temozolomide, cisplatin, chemotherapy, malignant, gliomas.

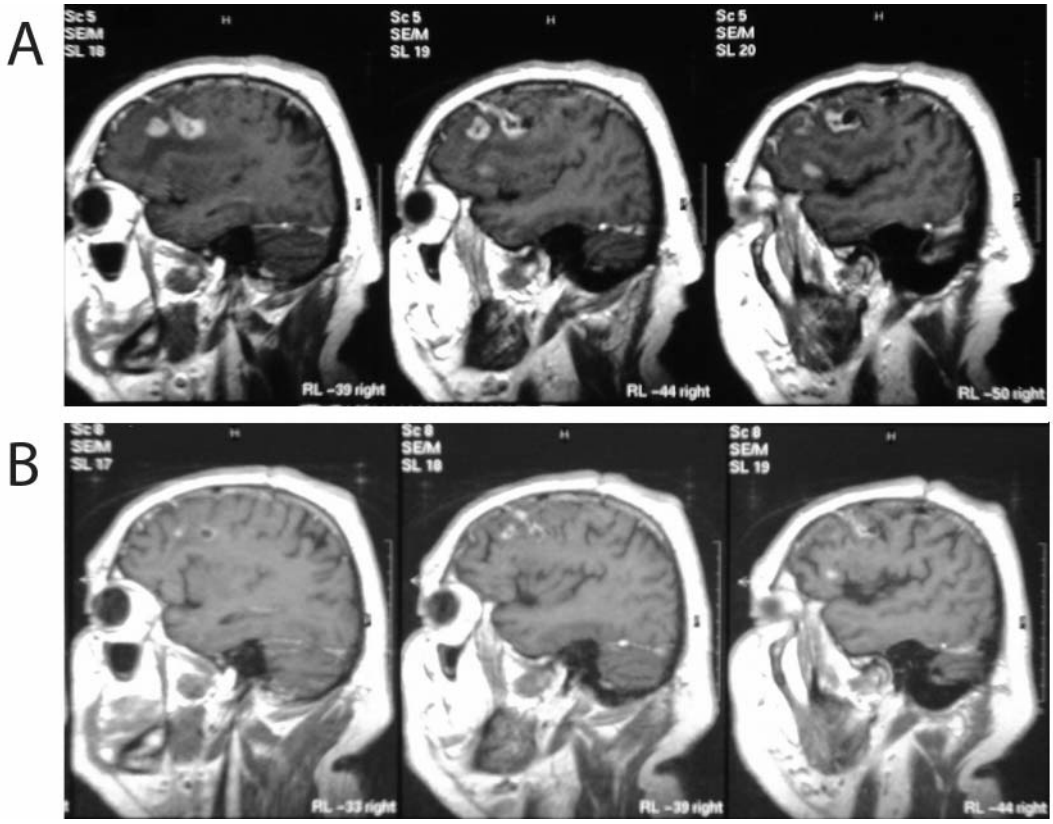

Figure 1. *Imaging of a patient with a partial response, before (A) and after (B) chemotherapy.*

seems to be due to alkylation of the *AGAT* gene promoter, whose region is rich in guanine-cytosine sequences with high cisplatin affinity. This is the basis for a synergistic effect between temozolomide and cisplatin.

The combination of cisplatin and temozolomide has already been tested in patients with relapsed glioblastoma as a first-line therapy, with an RR of 20.4% and a PFS-6 of 34% (7), but never in patients with temozolomide-refractory disease.

Patients and Methods

Patients. Heavily pre-treated patients with temozolomide-refractory and progressive high-grade malignant gliomas not eligible for further surgery and/or radiotherapy were enrolled into the study. Inclusion criteria were: histological or radiological diagnosis of high-grade malignant glioma, age between 15 and 75 years, ECOG performance status 0-3, more than 8 weeks of life expectancy, neutrophil count $>2.0\times10^9$/l and platelets $>100\times10^9$/l, serum creatinine and total bilirubin <1.5 × upper normal limit, possibility of regular follow-up. Patients with relevant concomitant illnesses or active infection in progress were excluded. Pregnant, and lactating patients were excluded and adequate contraception was strongly recommended for fertile female patients. Basal disease evaluation was performed within 3 weeks of beginning treatment. O^6-Methylguanine-DNA methyltransferase *(MGMT)* promoter methylation status was evaluated routinely using a methylation-specific polymerase chain reaction starting from formalin-fixed and paraffin-embedded tumour tissue.

Drug administration. Treatment was cisplatin at 75 mg/m^2 day 1 in combination with temozolomide at 150 mg/m^2 orally an hour before the evening meal of days 1 to 5. Cycles were repeated every 3 weeks. Standard antiemetic intravenous prophylaxis with 3 mg granisetron and 20 mg dexamethasone phosphate was performed before cisplatin administration, as well as intravenous infusion of 1275 ml/m^2 of 0.9% NaCl saline solution, with a supplement of 20 mEq KCl and 16 mEq $MgSO_4$, to prevent renal toxicity and restore K and Mg urinary loss.

Haematological toxicity in asymptomatic patients was evaluated the day before the administration of the subsequent chemotherapy cycle. Treatment was continued up to 6 cycles or disease progression. Patients with response to treatment or with stable disease after the sixth cycle continued treatment if it was well tolerated.

Patients and disease evaluation. Clinical and laboratory patient evaluation were performed before each chemotherapy administration. Disease evaluation was performed every two chemotherapy cycles using gadolinium nuclear magnetic resonance (NMR) scans (see Figure 1). Macdonald *et al*'s criteria were applied to evaluate response (7).

Table I. *Patient characteristics*.

Characteristic	No. of patients (n=24)
Gender	
Male	16
Female	8
Age (years)	
Median	57.6
Range	17.9-79.5
Histology	
Glioblastoma	16
MGMT	
Methylated	8
Not methylated	5
Not evaluable	3
AA grade III	2
ODG	3
OA	1
No biopsy	2
ECOG PS	
0	0
1	2
2	17
3	5
Prior therapy	
Surgery	22
Radiotherapy	23
Second surgery	7*
Carmustine wafers	3*
Chemotherapy other than temozolomide	7*

AA, Anaplastic astrocytoma grade III; ODG, oligodendroglioma, OA, oligoastrocytoma; *all patients with GBM histology.

OS was calculated from the start of the therapy with temozolomide and cisplatin until death. Time to progression (TTP) was defined as the time elapsed between the start of the treatment and documentation of progressive disease. The Kaplan-Meier method was used to estimate the survival curves. Analyses were performed using the GraphPad Prism statistical package (release 5; San Diego, CA, USA).

Results

Patient characteristics. From August 2004 to November 2008 24 consecutive patients were enrolled (Table I). Concomitant illnesses were not significant. Of the 18 patients with glioblastoma (GBM), 16 had undergone previous cytoreductive surgery and 17 had received radiotherapy; 7 also received a second-line chemotherapy (mostly fotemustine) after a second surgery and 3 had carmustine wafers (Gliadel^{R}) during the second surgery. All the 6 patients with a non-GBM histology (2 with anaplastic astrocytoma, 3 with oligodendroglioma and 1 with oligoastrocytoma) had cytoreductive surgery and radiotherapy.

Table II. *Overall toxicity during all treatment courses by type and grade*.

Type	No. of patients	
	Grade 1-2 n=24	Grade 3-4 n=24
Granulocytopenia	4	2
Anaemia	9	2
Thrombocytopenia	1	1
Anorexia	1	2
Nausea	6	1
Vomiting	9	1
Epigastralgia	2	1
Constipation	3	0
Somnolence	1	0
Fatigue	6	2
Myalgia	1	0
Arthralgia	1	0
Vascular venous	0	1
Uditive	1	0

Overall toxicity. All patients where valuable for toxicity. A total of 96 cycles were administered, with a median for each patient of 4 (range: 1-8). Toxicity (see Table II) was mostly of grade 1-2, haematological and gastroenterological. Eight patients complained of fatigue, which was of grade 3-4 in 2 patients. This symptom did not seem to be related to the anaemia but a direct consequence of the chemotherapy administration. Due to the grade 3-4 toxicity treatment-related episodes, 8 patients required a dose reduction to 75% or less of the planned dose.

Objective responses and survivals. Twenty-three out of 24 patients were evaluable for response. Among the 17 evaluable patients with GBM, 5 had a partial response (29.4%), 6 patients had stable disease (35.3%) and 6 had progression. The disease control rate was 64.7%. Among the 6 patients with an initial histological diagnosis of WHO grade III glioma, we observed 2 partial responses (for an oligodendroglioma and an anaplastic astrocytoma) and 4 disease stabilizations (for 2 oligodendrogliomas, an anaplastic astrocytoma and an oliastrocytoma) with a disease control rate of 100%, although this result is probably overestimated due to the slower growth and to the short interval (every 2 courses of chemotherapy, *i.e.* 6 weeks) between the basal NMR and the subsequent disease evaluation.

Among GBM patients, the median TTP was 3.8 months (95% confidence interval 2.4-6.8), PFS-6 was 28% and OS 7.0 months (95% confidence interval 4.8-11.0), as shown in Figure 2.

One patient with an oligoastrocytoma progressed after 2.5 months of treatment and had an OS of 5.0 months; one

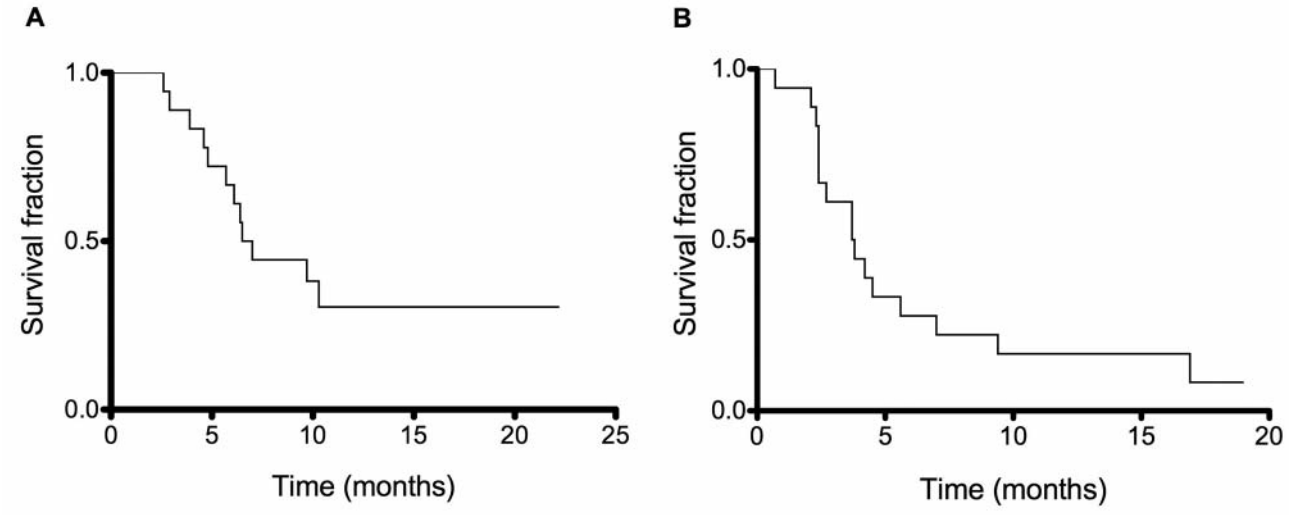

Figure 2. *Overall survival (OS) (A) and time to progression (TTP) (B) curves. Median OS=7.0 (95% CI 4.8-11.0); Median TTP=3.8 (95% CI 2.4-6.8).*

patient with an anaplastic astrocytoma was lost from follow-up and a patient with anaplastic oligodendroglioma had a TTP of 6.8 months and then was lost from follow-up. The other 3 patients with WHO grade III gliomas were not evaluable for TTP and OS after 5.5, 7.3 and 10.0 months of follow-up, respectively.

Discussion

In GBM patients we obtained interesting efficacy data, especially considering the heavily pre-treated and poor performance status of our study population (see Table I). The addition of cisplatin to temozolomide likely adds efficacy as compared to single-agent temozolomide and probably results in *in vivo* synergism between the two drugs as evidenced in the preclinical studies (6).

Brandes *et al.* (8) administered a four-week schedule of cisplatin and temozolomide to patients with relapsed GBM who had not received prior chemotherapy. They obtained partial responses in 20.4% of cases, with a PFS-6 of 34%. We obtained a higher partial response (30%) with a shorter PFS-6 (28%), probably due to the patient population of poor ECOG PS, heavy pre-treatment and temozolomide-refractory disease.

Some points might help to explain the higher RR that we observed. We administered cisplatin and temozolomide with a greater dose-intensity, 75 mg/m^2 every three weeks instead of 4 weeks, and in particular with a greater "synergistic intensity" between cisplatin and temozolomide, occurring every three weeks instead of every four weeks. On the other hand we have to consider that 2 patients who had a partial response progressed during temozolomide adjuvant treatment. TTP of these patients from the end of radiation was of 4.6 and 4.4 months, respectively which do not meet the Brandes *et al.* criteria in defining a pseudoprogression (9) but they had long TTP after response to the combination of cisplatin and temozolomide (16.9 and 19+ months). The hypothesis of pseudoprogression was thus not definitively excluded. Nevertheless, excluding these 2 patients from the analysis, the response rate remains 20%, the same RR obtained in first-line therapy by Brandes *et al.* (8), but the PFS-6 is reduced to 25% *vs.* 34% obtained in first-line therapy, which was to be expected considering that our patients were temozolomide refractory and heavily pretreated, with second surgery, placing of carmustine wafers and second-line chemotherapy (mostly fotemustine). For instance, in a small series of patients with similar characteristics that we treated with bevcizumab (data not published), we obtained a partial response of 14% (7 patients), which is far from the RR of 57% reported in a prior phase II study (4).

Of the 5 patients with unmethylated *MGMT* promoter, 2 obtained a partial response with TTP of 4.5 and 7.2 months, respectively. The addition of cisplatin to temozolomide might overcome the *in vivo* resistance to temozolomide of GBM with unmethylated *MGMT* promoter (10). Unfortunately, due to the small number of patients, a comparison between those with methylated and those with unmethylated *MGMT* promoter is not possible.

In an additional analysis, we compared the OS of patients aged 60 years. We found a statistically significant difference in favour of younger patients, who had an OS twice that of their older counterparts (Figure 3). This observation might help in selecting patients for further medical treatment considering that an intensive approach might help to reach a median OS from first diagnosis of

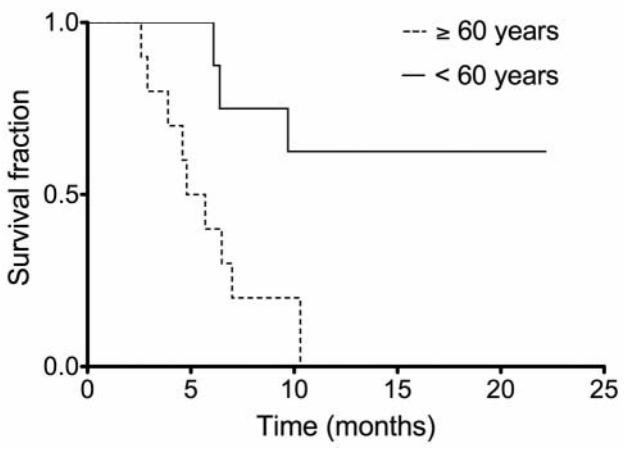

Figure 3. *Overall survival curves by age. Log-rank (Mantel–Cox) test: p=0.0034.*

about 20 months, as we obtained in this GBM patient series, with a median OS for any age of 20.6 months (95% confidence interval 15.7-not available).

In conclusion, adding cisplatin to temozolomide could be an option for patients no longer responsive to temozolomide alone and for those patients with unmethylated *MGMT* promoter, although a randomized study for *MGMT methylation*-negative patients should be designed to clarify this latter issue.

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