Temozolomide 3 weeks on and 1 week off as first-line therapy for recurrent glioblastoma: phase II study from gruppo italiano cooperativo di neuro-oncologia (GICNO)

The efficacy of temozolomide strongly depends on O\textsuperscript{6}-alkylguanine DNA-alkyl transferase (AGAT), which repairs DNA damage caused by the drug itself. Low-dose protracted temozolomide administration can decrease AGAT activity. The main end point of the present study was therefore to test progression-free survival at 6 months (PFS-6) in glioblastoma patients following a prolonged temozolomide schedule. Chemosensitive glioblastoma patients with disease recurrence or progression after surgery and standard radiotherapy were considered eligible. Chemotherapy cycles consisted of temozolomide 75 mg/m\textsuperscript{2}/daily for 21 days every 28 days until disease progression. O\textsuperscript{6}-methyl-guanine-DNA-methyl-transferase (MGMT) was determined in 22 patients (66.7%). A total of 33 patients (median age 57 years, range 31–71) with a median KPS of 90 (range 60–100) were accrued. The overall response rate was 9%, and PFS-6 30.3% (95% CI: 18–51%). No correlation was found between the MGMT promoter methylation status of the tumours and the overall response rate, time to progression and survival. In 153 treatment cycles delivered, the most common grade 3/4 event was lymphopenia. The prolonged temozolomide schedule considered in the present study is followed by a high PFS-6 rate; toxicity is acceptable. Further randomised trials should therefore be conducted to confirm the efficacy of this regimen.

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Glioblastoma multiforme (GBM), the most aggressive brain tumour in adults despite, advances in diagnosis and treatment made over the past two decades, is still incurable (Brandes, 2003). Every effort should, therefore, be made to develop new approaches that maximise an active drug, such as temozolomide (TMZ), which is the backbone in the treatment of brain tumours. However, the efficacy of this therapy is limited by the presence of intrinsic or acquired resistance mechanisms. Temozolomide exerts its activity by means of DNA methylation at the N\textsuperscript{7} and O\textsuperscript{6} position of guanine and at the O\textsuperscript{3} position of adenine (Denny et al, 1994). Although O\textsuperscript{6}-methylguanine accounts for few adducts formed by TMZ, it plays a critical role in the cytotoxicity of the drug. The DNA repair protein, O\textsuperscript{6}-alkyl-guanine-DNA-alkyltransferase (AGAT), encoded by the MGMT gene, reverts resistance to TMZ by removing cytotoxic methyl adducts from the O\textsuperscript{3} position of guanine. As AGAT removes methyl adducts from DNA via a suicide mechanism, it has also been suggested that protracted exposure to an alkylating agent may not only saturate AGAT copies available within cancer cells, but may also inactivate the new molecules while they are being synthesised, thus leading to AGAT ‘depletion’ and overcoming the inherent resistance of glioma cells.

Telch et al (2003) showed that mean AGAT activity decreased by 63% in peripheral blood mononuclear cells (PBMC) after 14 days of protracted TMZ treatment, and by 73% after 21 days; these low levels persisted up to day 28. In the present study, AGAT activity in PBMC has been used as a pharmacodynamic surrogate end point for AGAT depletion, although changes in AGAT activity in PBMC may not reflect changes in tumour tissues. A phase I study was performed using this TMZ schedule (3 weeks on/1 week off) in patients with advanced-stage solid malignancies (Denis et al, 2000). Dose-limiting toxicity was thrombocytopenia, the maximum-tolerated dose being 100 mg/m\textsuperscript{2}. However, as toxicity was detected only after two courses of treatment, no definitive conclusions could be drawn regarding the long-term toxicity of the regimen at this dosage. The aim of the present phase II study was therefore to evaluate the effect of a prolonged TMZ schedule of 75 mg/m\textsuperscript{2}/daily for 21 days every 28 days in relation to progression-free survival at 6 months (PFS-6), response, toxicity and any correlation with MGMT gene promoter methylation status, in patients with recurrent or progressive GBM.
MATERIALS AND METHODS

Eligibility

Criteria for eligibility were: histological diagnosis of GBM, age >18 years, Karnofsky Performance Score (KPS) ≥60, normal baseline counts for neutrophils ≥1500/μl and platelets ≥100 000/μl; transaminases and alkaline phosphatase levels ≤1.5 times the upper normal limits; bilirubin and creatinine levels ≤1.25 times the upper normal limits; previous surgery followed by standard radiotherapy (60 Gy/30 fractions). Unequivocal evidence of disease recurrence or progression at gadolinium-enhanced MRI neuroimaging was also required. Patients accrued had at least one enhancing measurable lesion with a diameter of ≥2 cm, evaluated at least 3 months after the end of radiotherapy. Brain imaging, performed within 2 days after surgery, showing residual disease with the above characteristics was mandatory for patients undergoing repeat surgery for recurrence. All patients accrued had been on a stable dose of corticosteroids for at least 2 weeks before initiation of therapy. Patients with childbearing potential were to use effective contraception. Pregnant or breastfeeding patients were ineligible, as were patients who had previously received cytotoxic therapy, presented active infection or other uncontrolled diseases, psychiatric disturbances and/or a history of cancer other than resected nonmelanoma skin cancer or carcinoma in situ of the uterine cervix.

Availability of tumour specimens to perform the assessment of MGMT promoter methylation status was required for all patients. The study, approved by the Institutional Review Boards of all participating centres, was conducted according to the principles of the Declaration of Helsinki and the rules of Good Clinical Practice. All patients signed a form giving their fully informed consent to participate.

Treatment regimen

All patients were given TMZ 75 mg/m²/daily for 21 days every 28 days. No dose escalation was allowed. Patients fasted for at least 2 h before, and 2 h after, TMZ administration. As continuous daily TMZ can cause leucopenia, potentially increasing the risk of opportunistic infections, patients received oral trimethoprim-sulphamethoxazole to prevent Pneumocystis carinii pneumonia if the lymphocyte count fell <500/μl (Kovacs and Masur, 2000). Antiemetic prophylaxis with metoclopramide or a 5-hydroxytryptamine3 antagonist was also given.

Dose modifications

Patients were closely monitored for toxicity throughout cycles, all adverse events being recorded and graded according to the common toxicity criteria of the National Cancer Institute, version 3.0. (http://ctep.cancer.gov/forms/CTCAEv3.pdf).

Haematology was performed weekly, while complete biochemistry was assessed once per cycle, preferably on day 28. Chemotherapy was given if neutrophils were ≥1500/μl, lymphocytes ≥500/μl and platelets ≥100 000/μl; otherwise treatment was delayed for a maximum of 3 weeks until adequate recovery. If blood counts analysed throughout 3 weeks were still unsatisfactory, treatment was stopped. In cases of ≥G3 haematological toxicity at nadir or reversible G3 nonhaematological toxicity (except for nausea/vomiting), TMZ was reduced by 25%. If G4 haematological or G3 nonhaematological toxicity reappeared notwithstanding dose reductions, or if any type of nonhaematological G4 toxicity was observed, chemotherapy was interrupted.

The use of growth factors in order to maintain high blood counts and to administer chemotherapy at fixed intervals was proscribed. Patients were kept at the lowest corticosteroid dosage allowed in relation to their neurological status.

Efficacy measures

Progression-free survival was evaluated from the start of chemotherapy to progression; median survival (MST) was calculated from the start of chemotherapy to death for any reason. In this intent-to-treat study, data on all registered patients were included in the statistical analysis.

PFS, PFS-6 and MST were calculated using the Kaplan–Meier method (Kaplan & Meier, 1958); differences in progression and overall survival (OS) were evaluated by the log-rank test for statistical significance.

Patients were evaluated for response using clinical and neurological examinations (performed monthly before each cycle) and MRI or CT neuroimaging performed every two cycles, or earlier if indicated, according to Macdonald’s criteria (Macdonald et al., 1990). Neurological status was assessed by considering signs and symptoms possibly correlated with progression, as compared to the previous examination; each variation in daily corticosteroids dosage was recorded.

Responses were confirmed as complete or partial if they were constant at subsequent scans obtained at least 4 weeks apart from each other. An independent central review of CT and MRI scans was made for patients achieving complete (CR) or partial (PR) response or stable disease (SD), evaluated by local investigators. Patients were withdrawn if they had progressive disease, unacceptable toxicity, or retracted their consent.

DNA extraction and methylation-specific polymerase chain reaction

DNA from 10 mm paraffin sections of cerebral lesion was modified by sodium bisulphite, which converts unmethylated cytosine to uracil, according to the procedure of Herman et al. (1996). Modified DNA was submitted for methylation-specific PCR (MSP) by a nested-PCR protocol (Pal misano et al., 2000). As the quality of DNA obtained from formalin-fixed paraffin-embedded tumour tissue affects the success rate of MSP, in some cases MGMT methylation status was determined by a different nested-MSP approach, with a first pair of primers to obtain smaller amplicons (129 bp), for which forward and reverse primers have been described (Pal misano et al., 2000; van Engeland et al., 2003).

Statistical analysis

The trial was a phase II study, with PFS-6 percentage as the main end point. According to the Minimax design (Simon, 1989), our study, with its sample size of n = 33, had a 5% probability of rejecting (α) the hypothesis of a PFS-6 10% (P) and a 90% probability of accepting (1-β) the hypothesis of a PFS-6 of 30% (P.). If two, or fewer of the first 22 patients were progression free at 6 months, PFS-6 would be considered <10% and the study terminated. Otherwise, the study would be completed, the accrual target being 33 patients. If six or fewer of the 33 patients were progression-free at 6 months, then no further investigation of the treatment regimen was considered warranted. PFS, PFS-6 and OS were calculated using the Kaplan–Meier method (Kaplan and Meier, 1958), and differences in progression and survival in relation to prognostic factors were evaluated with the log-rank test. Student’s 7 and the Mann–Whitney U-tests were used to analyse normally and non-normally distributed variables between groups. All calculations were performed using S-PLUS software (MathSoft Inc., Seattle, WA, USA).

RESULTS

Patient characteristics

From November 2003 to September 2005, 33 patients (13 females; median age 57, range 31–71 years; median KPS 90, range 60–100)
were enrolled; their characteristics are reported in Table 1. All patients were evaluated for drug activity and toxicity.

**Methylation specific PCR analysis**

Methylation specific PCR (MSP) analysis was performed in all 33 patients enrolled in the trial. However, results were evaluable only in 22 patients, with an MSP success rate of 66%; this finding is comparable to those reported by other authors (Hegi et al, 2005). Among 22 patients for whom MSP was evaluable, 10 (45.5%) presented MGMT promoter methylated and 12 unmethylated (54.5%) status. No differences were found between patients with MGMT promoter methylated and those with unmethylated status for age ($P = 0.16$), gender ($P = 0.39$), performance status (PS) ($P = 0.72$), time-intervals between surgery and start of chemotherapy ($P = 0.21$), or between the end of radiotherapy and start of chemotherapy ($P = 0.15$). All patients had undergone one surgical procedure and full-dose radiotherapy, completed at least 3 months previously, but none had been given cytotoxic or cytostatic drugs.

**Progression-free survival**

All patients were followed-up to disease progression. The percentage of patients without progression at 6 months (PFS-6) was 30.3% (95% CI: 18–51%). Overall, the median PFS was 16.1 weeks (95% CI: 12.4–27.3), 15.6 weeks (CI: 11–NA) and 11.9 weeks (CI: 10.3–31.6) in patients with MGMT promoter methylated status and in patients with unmethylated MGMT promoter status, respectively. No significant differences were found between PFS, evaluated using the log-rank test, in relation to age ($P = 0.42$), KPS ($P = 0.77$), type of surgery ($P = 0.58$) and MGMT promoter methylated or unmethylated status ($P = 0.86$).

**Response**

Among the 33 evaluable patients, one CR (3%) and two PR (6%) were obtained, with an overall response rate of 9% (CI: 0–18.8%).

**Table 1** Characteristics of patients

| Characteristic                      | No. of patients (%) |
|------------------------------------|---------------------|
| **Gender**                         |                     |
| Female                             | 13 (39)             |
| Male                               | 20 (61)             |
| **Age (years)**                    |                     |
| Median                             | 57                  |
| Range                              | 31–71               |
| **KPS**                            |                     |
| Median                             | 90                  |
| Range                              | 60–100              |
| **Extent of resection**            |                     |
| Gross total resection              | 13 (39)             |
| Partial resection or biopsy        | 20 (61)             |
| **Previous treatment**             |                     |
| Primary resection                  | 33 (100)            |
| Radiotherapy                       | 33 (100)            |
| Chemotherapy                       | 0                   |
| Repeat surgery for recurrence      | 1                   |
| **Cycles of temozolomide**         |                     |
| $<3$                               | 18                  |
| 4–6                                | 7                   |
| 7–9                                | 6                   |
| $>10$                              | 2                   |

Seventeen patients had SD (51%; CI: 34.4–68.6%). All radiological responses were confirmed by an independent centralised review, and stable or decreased steroid dosage was confirmed in all patients on recording responses, which had a median duration of 30.4 weeks, the median duration of disease stabilisation being 25.6 weeks. No correlations were found between response to therapy and age ($P = 0.32$), KPS ($P = 0.96$), time-interval between surgery ($P = 0.19$) or end of radiotherapy ($P = 0.38$), MGMT promoter methylated or unmethylated status ($P = 0.63$) in responders (CR + PR) and SD patients vs PD patients.

**Overall survival**

Median survival as from the start of chemotherapy was 40 weeks (CI: 31–63), although this outcome may have been influenced by second-line treatments, 12 patients being treated with nitrosourea-based regimens, 3 with carboplatin and etoposide, 3 with new experimental drugs, and 15 with no other treatments. In all, 73% (CI: 59–90%) and 38% (CI: 24–59%) of the patients were alive at 6 and 12 months, respectively. Median survival and percentage of patients alive at 1 year in cases with methylated MGMT promoter status were 48.2 weeks (CI: 29–NA) and 50% (95% CI: 27–93%), respectively; in patients with unmethylated MGMT promoter status, these figures were 34.7 weeks (95% CI: 30–64.1) and 21.4% (CI: 8–58%), respectively. Only KPS (<90 vs $\geq$90) was related to survival ($P = 0.05$) (Figure 1).

**Toxicity**

A total of 153 treatment cycles were given to 33 patients, the median number of cycles per patient was three (range 1–15). The most common adverse event was lymphopenia (Table 2). As the cycles were delivered, a clear trend toward an increase in lymphopenia was observed, being present in 30% of patients during the first three cycles, and in 55% of the patients in the subsequent cycles. Although none of the patients had pneumocistis carinii pneumonia, five (13.6%) had grade 1–3 infections (one herpes zoster, one urinary infection and three upper respiratory tract infection) associated with lymphopenia grade 1 (one patient), grade 2 (two patients) and grade 3 (two patients). The most commonly found nonhaematological symptoms and signs of toxicity were constipation and grade 1–3 asymptomatic transaminase increase, observed in seven patients (21.2%). Eight cycles (5.2%) were delayed for a median time of 1 week (recovery...
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by several factors: chemotherapy administered at relatively low
6 of 30.3% was obtained. Our findings may have been influenced
continuous TMZ schedule for 21 days every 28 days, and a PFS-
correlation between MGMT cells during chemotherapy (Gerson, 2004) a search was made for a
gene, might be implicated in the protection of haematopoietic stem
tumour samples, and not in blood, thus indicating only an indirect
ever, MGMT promoter methylation status was determined on
tumour samples, and not in blood, thus indicating only an indirect
correlation between MGMT and toxicity. Grade 3–4 leukaemia was observed in three patients with methylated MGMT promoter
and in one patient with unmethylated MGMT promoter, lymphopaenia grade 3 was observed in four patients with methylated
MGMT promoter, and in one with unmethylated MGMT promoter.

DISCUSSION

In GBM patients with first recurrence, TMZ is active at a standard
single daily dose of 150–200 mg/m², administered for five days
every 4 weeks. In the three available studies investigating this regimen in this setting, PFS-6 was 21% (Yung et al, 2000) (CI:13–
29%), 18% (Brada et al, 2001) (CI:11–24%), and 24% (Brandes et al, 2002) (CI:14–42%). As the antitumour activity of TMZ
depends on the level of AGAT within tumour cells, several trials have
aimed to deplete AGAT via a continuous dosing schedule (Tolcher et al, 2003). In their phase II study with continuous TMZ
administration at a dosage of 75 mg/m²/day over a 6-week period
with 4 weeks’ rest, in 28 pretreated GBM patients, Khan et al
achieved a PFS-6 of 19%, a median PFS 2.3 months and zero
responses. This schedule thus allowed a higher dose intensity
(1260 mg/m²) over 4 weeks than the standard schedule (1000 mg/m²)
with good tolerability, although the results did not support its use
in patients with recurrent GBM. Wick et al (2004) treated 21 GBM
patients with a different 28-day period continuous schedule (TMZ
at 150 mg/m² delivered for 1-week on/1-week off) with a drug
intensity of 2100 mg/m² and AGAT (Tolcher et al, 2003) was
depleted: a PFS-6 of 48% was obtained (43% patients were
pretreated with 1–2 chemotherapy regimens) with modest toxicity.

However, on using the same regimen, Chinot et al (2005) obtained
a PFS-6 of only 21% in 29 inoperable previously untreated GBM
patients.

We treated recurrent chemonaive GBM patients with a continuous
TMZ schedule for 21 days every 28 days, and a PFS-6 of 30.3% was obtained. Our findings may have been influenced
by several factors: chemotherapy administered at relatively low
doses while following a frequent metronomic schedule may have
optimised the antiangiogenic effect of cytotoxic agents, providing
better results than an intensified weekly schedule (Kurzen et al,
2003). Moreover, a high-dose intensity (1575 mg/m²) was achieved
and AGAT depletion was more prolonged than in a weekly schedule, in which recovery of AGAT activity begins immediately
after 7 days (Tolcher et al, 2003).

The results reported in studies on MGMT gene promoter status
are controversial. It has been maintained that MGMT promoter methylation assessed by MSP is associated with a longer survival in
GBM patients treated with radiotherapy and TMZ (18.2 vs 12.2
months, P <0.001) (Hegi et al, 2005). However, Paz et al (2004)
reported that MGMT promoter methylation was correlated with
response to standard TMZ, but not with OS. Findings made using
alternative methods (immunohistochemistry or activity tests) are
contradictory, some confirming (Friedman et al, 1998) and others
denying (Middleton et al, 1998) a relationship with time to
gression or response to TMZ.

To our knowledge, ours is the first trial to study correlations
between MGMT promoter methylation status, assessed by MSP,
and treatment outcome with a continuous TMZ schedule. Findings
for/MSP were available in 22 (66.7%) of the 33 patients enrolled in
the study; this percentage is similar to those reported (59–67%)
in MSP studies made by other authors (Hegi et al, 2004; Hegi et al,
2005). In our study, an overall median PFS of about 4 months and
a PFS-6 of 30.3% were obtained: these results appear superior to
those obtained with the standard TMZ schedule. In 22 patients for
whom MGMT promoter status was available, no significant
difference was found between MGMT promoter methylated or
unmethylated patients for median PFS and PFS-6 (15.6 weeks and
20% vs 11.9 weeks and 21.4%). This suggests that AGAT depletion
achieved with protracted TMZ increases the sensitivity of
unmethylated tumours to TMZ, leading to an increase in PFS and
OS which would have been less than that obtained by us if
these patients had been treated with the traditional TMZ schedule.
However, this observation, based on a small number of patients,
should be demonstrated by findings from prospective studies with
patients stratified according to methylation status, as in the
ongoing phase III RTOG/EORTC study.

The standard TMZ schedule (Brada et al, 2001; Yung et al, 2000)
incurs higher grade 3/4 thrombocytopenia (7–10% vs 3) and lower
grade 3/4 neutropenia (4–4.5% vs 12) rates than our 3-week on/1-
week off schedule. Cumulative lymphopaenia was observed in
45.4% of our patients, this outcome being comparable to those
reported by other authors following other protracted schedules
(Brock et al, 1998; Khan et al, 2002; Su et al, 2004; Wick and
Weller, 2005). This high incidence of lymphopaenia may have been
due in part to the concomitant administration of steroids: 75% of
patients received both steroids and continuous TMZ. The standard
TMZ schedule rarely leads to lymphopaenia, no such cases being
reported in 250 GBM patients treated in phase II–III trials (Yung
et al, 2000; Brada et al, 2001).

Although the number of severe haematologic events observed by
us may be of limited statistical power, we observed an association

Table 2  Toxicity per patient

| Grade 1 no. of patients (%) | Grade 2 no. of patients (%) | Grade 3 no. of patients (%) | Grade 4 no. of patients (%) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Neutropenia                 | 1 (3)                       | 2 (6)                       | 2 (6)                       | 2 (6)                       |
| Lymphopaenia                | 1 (3)                       | 6 (18.2)                    | 8 (24.2)                    | 0                           |
| Thrombocytopenia            | 1 (3)                       | 0                           | 0                           | 1 (3)                       |
| Anemia                      | 5 (15.2)                    | 1 (3)                       | 1 (3)                       | 0                           |
| Nausea                      | 0                           | 2 (6)                       | 1 (3)                       | 0                           |
| Constipation                | 4 (12.1)                    | 4 (12.1)                    | 1 (3)                       | 0                           |
| Increased transaminase      | 5 (15.2)                    | 1 (3)                       | 1 (3)                       | 0                           |
| Infection with lymphopaenia | 1 (3)                       | 3 (9.1)                     | 1 (3)                       | 0                           |

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between grade 3 lymphopenia, grade 4 haematological toxicity and MGMT status. Based on experimental evidence indicating low AGAT activity in bone marrow CD34 cells (Gerson et al, 1996), it may be suggested that haematological progenitors are especially sensitive to strategies that inactive AGAT: when O6 benzylationguanine, an AGAT specific inhibitor, is added to BCNU, haematological toxicity increases (Friedman et al, 2000). A correlation between AGAT activity in PBMC and haematological toxicity has been demonstrated by Tolcher et al (2003) in patients treated with a prolonged schedule. However, in our study MGMT promoter methylation status was assessed only on tumour samples, and MGMT promoter methylation may vary among different tissues. Based on these considerations it would be interesting to conduct a prospective trial studying MGMT expression in blood and tumour samples in order to understand the activity of temozolomide and its toxicity profile as this would be conducive to personalising drug delivery.

In June 2005, TMZ concomitant with radiotherapy followed by six cycles of maintenance chemotherapy became standard therapy worldwide (Stupp et al, 2005). The present study will therefore probably be the last classic phase II trial to be performed using an alternative TMZ schedule as first-line chemotherapy in chemo-naïve and recurrent patients after standard radiotherapy. The findings made indicate that a phase III trial on a larger series should be launched in order to evaluate whether new chemo-

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