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Safety and pharmacokinetics of temozolomide using a dose-escalation, metronomic schedule in recurrent paediatric brain tumours

S. Baruchel[a,*], M. Diezi[a], D. Hargrave[a], D. Stempak[a], J. Gammon[a], A. Moghrabi[b], M.J. Coppes[c], C.V. Fernandez[d], E. Bouffet[e]

[a]New Agent and Innovative Therapy Program, Department of Paediatrics, Division of Haematology/Oncology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ont., Canada M5G 1X8
[b]Hôpital Ste Justine, Division of Haematology Oncology, Université de Montréal, Canada
[c]Departments of Oncology and Paediatrics, University of Calgary, Calgary, Alb., Canada
[d]IWK Health Centre and Dalhousie University, Halifax, Nova Scotia, Canada
[e]Paediatric Brain Tumour Program, The Hospital for Sick Children, Toronto, Ont., Canada

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ABSTRACT

The aims of this study were to determine the maximum tolerated dose (MTD), toxicity and pharmacokinetics of oral temozolomide administered over 42 d in children with recurrent/refractory brain tumours. Cohorts of 3–6 patients were treated for 42 d, followed by a 7-d rest period for a maximum of 6 cycles. Patients were stratified as heavily pre-treated (HPT) and non-heavily pre-treated (NHPT). Starting doses were 50 mg/m^2 (HPT) or 75 mg/m^2 (NHPT). Out of 28 patients enrolled, 20 were evaluable for toxicity and 19 for pharmacokinetics. Three patients in the NHPT group developed grade 3/4 haematological toxicity, 2 experienced dose-limiting toxicity (thrombocytopenia) at 100 mg/m^2, and 9/20 developed grade 3 lymphopenia. MTD in both strata was 85 mg/m^2. Responses were observed in 4 patients: 2 complete responses (CR) in medulloblastoma and supratentorial primitive neuroectodermal tumours (PNET), and 2 partial responses (PR) in high-grade glioma, respectively. Overall cumulative exposure was at least 1.5 times higher than in the 5-d administration schedule. In conclusion, the recommended dose of temozolomide is 85 mg/m^2 × 42 d. Dose-limiting toxicities are thrombocytopenia and lymphopenia. The observed response rate warrants phase II studies.

1. Introduction

Temozolomide (Temodal®, Temodar®, Schering-Plough; TMZ) has shown activity against a wide range of tumours in early pre-clinical studies, including leukaemia, lymphoma and solid tumours, such as melanoma, sarcoma, lung carcinoma, glioblastomas and astrocytomas.[1] Such activity was demonstrated to be schedule-dependent, with repeated doses being more effective than single doses. TMZ has an excellent oral bioavailability.[2] It distributes extensively into the tissues and has good penetration in the central nervous system (CNS), which is potentially increased with concomitant use of radiotherapy.[3,4]

In adults, TMZ has been used in a variety of tumours, as a single agent or in combination, and with different administration schedules.[5–9] Phase I/II studies of TMZ showed promising results in adult gliomas.[10–15] Yung and colleagues

* *Corresponding author:* Tel.: +1 416 813 7795; fax: +1 416 813 5327.
E-mail address: sylvain.baruchel@sickkids.ca (S. Baruchel).
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showed a 21% 6-month progression-free survival (PFS) in adult patients with relapsed glioblastoma multiforme treated with TMZ compared with an 8% PFS in similar patients treated with procarbazine.[13] In a phase II trial of TMZ given over 5 d in low-grade glioma, Quinn and colleagues described a 6-month and 12-month PFS of 98% and 76%, respectively.[14] Stupp and colleagues recently reported a statistically significant and clinically meaningful benefit of the association of TMZ and radiotherapy in adult patients with glioblastomas.[16]

Pre-clinical studies of TMZ in paediatric solid tumour xenograft models (neuroblastomas, rhabdomyosarcomas and brain tumours) showed encouraging results, especially in cell lines expressing low levels of O^6-alkylguanine-DNA alkyltransferase (AGT), the DNA-repairing enzyme implicated in TMZ resistance, which is encoded by the gene *MGMT*.[17–19] Inactivation and depletion of this enzyme seems to be related to prolonged survival of patients, as pointed out by Hegi and colleagues.[19] Protracted administration of TMZ, even at relatively low daily doses, leads to significant and prolonged depletion of AGT activity, which may enhance the anti-tumour effect of the agent.[20]

In a phase I study of TMZ given over 5 d every 28 d in children with advanced cancer, Estlin and colleagues showed myelosuppression (especially thrombocytopenia) to be the dose-limiting toxicity.[21] In this study, 2 out of 5 patients with high-grade astrocytomas responded to TMZ and 1 patient had stable disease.[21] Their data suggested that 1000 mg/m^2 per 5-d cycle can be used for phase II studies in patients who did not receive prior craniospinal irradiation or nitrosoureas. An intergroup phase II study by the United Kingdom Children's Cancer Study Group and the Société Française d'Oncologie Pédiatrique using the same 5-d regimen in children with recurrent malignant gliomas and diffuse brain-stem glioma reported a lower objective response rate.[22]

Pre-clinical evidence has shown that, by increasing the frequency of administration of lower doses of chemotherapy, endothelial cells can be targeted, thereby resulting in an anti-cancer effect through an anti-angiogenic mechanism.[23–25] This low and increased frequency of administration is called metronomic (LDM) chemotherapy.[26,27] Metronomic administration of TMZ (100 mg daily × 42 d) has been used in adults demonstrating an overall increase in drug exposure and inducing a more continuous suppression of AGT.[20] In a phase I trial of oral continuous low-dose TMZ over a 6–7-week period in adult patients with refractory solid tumours (the majority of which were gliomas), Brock and colleagues reported a dose-limiting toxicity (DLT) of 100 mg/m^2/d, with the main toxicity being myelosuppression with thrombocytopenia. TMZ administration of 75 mg/m^2/d over a 7-week period permits a 2.1-fold greater drug exposure per 4 weeks in comparison with the 5-d schedule of 200 mg/m^2/d repeated every 28 d. The overall response rate was 33% (glioma patients, 41% and a further 25% had stable disease).[28] A phase II study using the same type of schedule in 35 adult patients with recurrent gliomas concluded that metronomic TMZ was well tolerated, but failed to show an improved rate of response or survival.[29] However, two small paediatric series using a metronomic TMZ administration (90 mg/m^2 daily × 42 d) with or without radiotherapy in brain tumours patients showed very encouraging results.[30,31]

We present here the results of a phase I and pharmacokinetic study of continuous low-dose oral TMZ in paediatric patients with recurrent brain tumours. TMZ was given in courses of 42 d, with rest intervals ranging from 1 to 3 weeks between courses.

2. Patients and methods

This study was a Canadian, multicentre clinical trial. The study was approved by each Institutional Research Ethics Board and informed consent was obtained from parents or guardians prior to study enrolment. Assent was obtained from children over 7 years of age, where possible.

2.1. *Patient eligibility*

Patients had to be less than 18 years of age, with a recurrent or progressive brain tumour that was refractory to standard therapy, or for which there was no standard salvage treatment. The Karnofsky performance status had to be greater or equal to 60. Subjects had to be on a stable dose of steroids for at least 1 week prior to enrolment, and have a life expectancy of more than 12 weeks. Patients also had to have recovered from any toxic effects of previous chemotherapy, and be off growth factors for at least 1 week. A haemoglobin count greater than 100 g/l, a platelet count higher than 100×10^9/l and a neutrophil count greater than 1.5×10^9/l were mandatory, as was creatinine, urea, total and direct bilirubin lower than 1.5 times the upper limit of laboratory normal and AST and ALT lower than 3 times normal. Patients were not eligible if they had undergone bone marrow transplantation within the prior 6 months. Females of reproductive potential had to have a negative pregnancy test.

Exclusion criteria included previous treatment with dacarbazine (DTIC) or TMZ. Also excluded were patients with frequent vomiting or any other medical condition interfering with oral intake that would prevent the patient from swallowing TMZ capsules. Patients requiring a dose delay of more than 4 weeks between courses or a dose reduction to less than 50 mg/m^2 were taken off the study. Patients were allowed to resume a subsequent cycle at the same dosage if laboratory values were returned to the level of study entry criteria; a dose reduction schema was established according to Common Toxicity Criteria (CTC) version 2.0, NCI, April 30th, 1999.[32]

2.2. *Study design*

This was a phase I study designed to determine the maximum tolerated dosed (MTD), safety and pharmacokinetics of TMZ given as a continuous oral regimen in this paediatric patient population. Response analysis was a secondary end-point and was reported on intent to treat. Patients were stratified according to their tumour diagnosis and prior therapy: stratum A (heavily pre-treated (HPT)) versus stratum B (non-heavily pre-treated (NHPT)). HPT patients were defined as those who had received more than two chemotherapy regimens or high-dose chemotherapy with stem cell rescue prior to enrolment. Patients who received cranio-spinal irradiation were also considered as HPT. All other patients were stratified

as NHPT. A starting TMZ dose of 75 mg/m^2 was used in NHPT patients (80% adult MTD), while the starting dose for HPT patients was 50 mg/m^2 for safety reasons. No intra-patient dose escalation was allowed. DLT was defined as the dose causing a greater than or equal to grade 3 renal, cardiovascular, pulmonary or CNS toxicities or any grade 4 non-myelotoxicity or any grade 4 myelotoxicity lasting longer than 1 week, or severe infection. Only the first course was considered for the evaluation of DLT and MTD. Toxicity data are, however, collected to document chronic toxicity.

Patients were enrolled in cohorts of three. If none of the three patients had DLT, the dose was escalated to the next level. If one of the three patients in a cohort had DLT, three more patients were enrolled at this dose level. If none of these three additional patients had DLT, the dose was escalated by 30% to the next level. If DLT was noted in two of three to six patients at any given dose level, the MTD was exceeded and three more patients were treated at the next lower dose level, provided only three patients had been treated previously at that level. The MTD was defined as the dose level immediately below the dose level at which two of three to six patients experienced DLT. A 25% dose reduction strategy was established based on occurrence of toxicity. Toxicity was assessed according to the Common Toxicity Criteria[32] on an ongoing basis throughout the study period. Routine, weekly haematology and biochemistry blood work was performed. The 1–3-week rest periods between cycles allowed for bone marrow recovery. Therapy was restarted when the absolute neutrophil count (ANC) recovered to 0.5×10^9/l and platelet count was equal or greater than 100×10^9/l. A delay in the administration of the next cycle of more than 4 weeks due to lack of bone marrow recovery or any grade 3 or 4 non-haematological toxicity was considered to be a serious adverse event and the patient was withdrawn from the study. Subsequent cycles were administered for up to 1 year for a total of six cycles. Because of reports of *Pneumocystis carinii* pneumonia (PCP) in patients receiving the combination of TMZ and steroids,[30,33] sulphamethoxazole/trimethoprim prophylaxis was offered to all patients on steroids. Prophylactic treatment for nausea and vomiting was provided with 5-HT$_3$ receptor antagonists. Patients received platelets transfusion when platelet counts dropped below 50,000/mm^3.

2.3. Drug formulation and administration

TMZ was administered orally to fasting patients once daily in the morning for 6 weeks at the described doses. Temodal® was supplied by Schering Plough (Pointe Claire, Québec, Canada). Opening of capsules was not permitted and doses were rounded up to the nearest 5 mg. Patients were instructed to swallow the whole capsules in rapid succession and not to chew them. If vomiting occurred during the course of treatment, no re-dosing occurred.

2.4. Patient evaluation

Patients were evaluated before each cycle with a physical examination that included a neurological evaluation, a neurological performance grading and a Karnofsky performance status. Radiological tumour assessment was performed by either gadolinium magnetic resonance imaging (MRI) or contrast-enhanced computed tomography (CT) scan on the last scheduled day of each cycle and response was evaluated using bi-dimensional measurement as followed: radiological complete response (CR) was defined as disappearance of all enhancing tumour for at least 4 weeks, partial response (PR) as a greater than 50% reduction in enhancing tumour, and progressive disease (PD) as a greater than 25% increase in tumour size. All other situations were defined as stable disease (SD). If the cerebro-spinal fluid contained malignant cells at diagnosis, a lumbar puncture was repeated before each cycle.

2.5. Pharmacokinetic studies

Pharmacokinetic studies were conducted on day 1 of the first cycle. Samples were collected immediately before and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after the TMZ dose. For each time-point, 5 ml of whole blood was collected from a central venous line or from a peripheral venous line into pre-chilled (4 °C) lithium heparin Vacutainer® tube and placed on ice. Within 10 min of collection, blood samples were centrifuged at 4 °C. The resulting plasma was acidified with 0.1 ml of 8.5% phosphoric acid and frozen at −80 °C until analysis. TMZ was extracted from the plasma samples using liquid–liquid extraction and analysed by high performance liquid chromatography (HPLC) with ultraviolet (UV) light detection as described by Estlin and colleagues.[21]

2.6. Pharmacokinetic analysis

C_{max} and T_{max} were determined for each patient by visual inspection of the plasma concentration versus time curve. Area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$), apparent terminal disposition rate constant (k_e) and terminal elimination half-lives ($t_{1/2}$) were estimated from the concentration–time data using no compartmental techniques (WinNonlin Professional v. 4.0.1, 1998–2002; Pharsight Corporation). Apparent oral clearance (Cl/F) and apparent oral volume of distribution (V_d/F) were calculated using the formulas $Cl/F = dose/AUC_{0-\infty}$ and $V_d/F = dose/(k_e \cdot AUC_{0-\infty})$, respectively, and these values were normalised for body surface area.

3. Results

3.1. Patient characteristics and treatment

Twenty-eight patients were enrolled on the study between February 2000 and August 2003. Twenty-seven patients (14 females and 13 males) were eligible, 1 patient was subsequently found ineligible because she did not have a recurrent brain tumour but gliomatosis cerebri secondary to bone marrow transplant/total body irradiation (BMT/TBI) and was reported separately.[36] Table 1 lists the patients' baseline characteristics. Patients could be categorised into 6 diagnostic categories, namely high-grade glioma (6, 22.2%), primitive neuroectodermal tumour (PNET)/medulloblastoma (7, 25%, 6 posterior fossa medulloblastoma and 1 supratentorial PNET) low-grade gliomas (5, 18.5%), brain-stem gliomas (4, 14.8%), ependymoma (4, 14.8%) and meningioma (1, 3.7%). Patients were

Table 1 – Patients' characteristics

	Heavily pre-treated (HPT) (n = 15)	Not heavily pre-treated (NHPT) (n = 12)	All patients (n = 27)
Age (years), median (min, max)	9 (4,19)	12.5 (5,19)	10 (4.0,19.0)
Gender, n (%)			
Male	9	4	13 (48.1%)
Female	6	8	14 (51.9%)
Diagnosis			
Low-grade gliomas	4	1	5 (18.5%)
High-grade gliomas	3	3	6 (22.2%)
PNET/medulloblastoma	6	1	7 (25.9%)
Brain-stem glioma	0	4	4 (14.8%)
Meningioma	0	1	1 (3.7%)
Ependymoma	2	2	4 (14.8%)
Prior therapy*, n (%)			
CM/RT/Surg	10	8	18 (66.7%)
CM/RT	1	3	4 (14.8%)
CM/Surg	3	0	3 (11.1%)
RT/Surg	0	1	1 (3.7%)
CM only	1	0	1 (3.7%)
Prior chemotherapy regimens, n (%)			
0	0	2	2 (7.4%)
1	4‡	9	13 (48.1%)
2	5‡	1	6 (22.2%)
3	6	0	6 (22.2%)
Prior CSI history, n (%)			
No	9	10	19 (70.4)
Yes	6	2§	8 (29.6)

* CM, chemotherapy; RT, radiation; Surg, surgery; CSI, craniospinal irradiation; PNET, primitive neuroectodermal tumour.
‡ Nine patients with less than 3 prior regimens have been classified as HPT due to other considerations: 5 patients had CSI, 2 patients received radiation therapy equivalent to CSI, 1 had cranial radiation and 2 additional surgeries and 1 had two regimens of chemotherapy for previous acute lymphoblastic leukaemia (ALL).
§ Classified as NHPT instead of HPT because the decision to classify CSI as a determinant of HPT was made after the patients were enrolled.

prospectively stratified according to their prior treatment, as described above: 15 were heavily pre-treated (HPT) and 12 not heavily pre-treated (NHPT). Two patients who received cranio-spinal irradiation (CSI) were classified as NHPT instead of HPT because the decision to classify CSI as a determinant of HPT was made after the patients were enrolled.

Table 2 lists the daily dose escalation and the development of DLT. The starting dose in the NHPT stratum was 75 mg/m^2 and the DLT was defined as 100 mg/m^2/d. The starting dose in the HPT stratum was 50 mg/m^2/d and the highest dose administered in this group was 85 mg/m^2/d. A further dose escalation in the HPT cohort was judged unethical as the DLT was already established in the NHPT cohort at 100 mg/m^2/d. Seven patients were not evaluable for toxicity because they did not complete the first cycle of treatment due to rapid disease progression.

Table 2 – Daily dose of temozolomide (TMZ) and development of dose-limiting toxicity (DLT) (cycle 1)

	Dose (mg/m^2)	n	DLT
Heavily pre-treated	50	5 (2)*	0
(n = 15)	65	3 (0)	0
	85	7 (1)	0
Total		15 (3)	0
Not heavily pre-treated	75	7 (2)	0
(n = 12)	100	5 (2)	2§ (0)
Total		12(4)	2§ (0)

* Numbers in parentheses are the numbers of patients who were not evaluable.
§ Both patients had grade 4 thrombocytopenia.

Seven patients (25.9%) completed at least six cycles. The remaining 20 patients did not complete the six-cycle study because of disease progression and death (18 patients) or severe adverse events (2 with prolonged thrombocytopenia). There were no toxicity-related deaths. The total number of cycles administered was 85, 6 of 27 patients did not complete cycle 1. The mean number of cycles per patient was 3.2, with a minimum of 1 and a maximum of 13. Table 3 summarises the number of cycles delivered for each stratum.

3.2. *Toxicity of TMZ*

Table 4 summarises the most frequently observed haematological and non-haematological toxicities. From the myelotoxicity point of view, a total of 9/20 evaluable patients developed grade 3 lymphopenia (<0.5 10^9/l according to CTC), 5 out of 12 in the HPT group and 4 out of 8 in the NHPT group. In all 5 HPT patients, lymphopenia occurred after more

Table 3 – Summary of the treatment delivered

Number of cycles delivered	Heavily pre-treated (HPT) (n = 15)	Not heavily pre-treated (NHPT) (n = 12)	All patients, n (%) (n = 27)
1†	8	8	16 (59.3)
2	0	1	1 (3.7)
4	2	0	2 (7.4)
5	1	0	1 (3.7)
6	1	2	3 (11.1)
>6	3	1	4 (14.8)

† Including less than 1 cycle.

Table 4 – Observed grade 3/4 toxicities, all cycles

	NHPT n (%)	HPT n (%)	All patients
Haemoglobin	1 (13)	0	1 (5)
Leukocytes	3 (38)	1 (8)	4 (20)
Lymphocytes	4 (50)	5 (42)	9 (45)
Neutrophils	2 (25)	0	2 (10)
Thrombocytes	3 (38)	0	3 (15)
HSV infections	2 (25)	2 (17)	4 (20)
Abdominal pain	0	1 (8)	0
Anorexia	2 (25)	0	2 (10)
Vomiting	1 (13)	2 (17)	3 (15)

HSV, Herpes simplex virus; HPT, heavily pre-treated; NHPT, not heavily pre-treated.

than two cycles of TMZ. Lymphocyte count recovery was observed in only 3 of these 5 HPT patients by the end of the data collection period. In the NHPT cohort, the 4 patients developed additional grade 3/4 haematological toxicities after the first cycle of treatment, including thrombocytopenia (n = 3), leucopaenia (n = 2) and anaemia (n = 1). Following subsequent TMZ cycles, these toxicities became slightly more severe, with 1 additional patient in the NHPT group progressing to grade 3/4 myelosuppression.

Three patients developed thrombocytopenia (2 patients in the NHPT group developed DLT at a dose of 100 mg/ m^2), 1 patient did not recover at the end of cycle 1 and remained below dose 50,000/mm^3 at week 12, therefore this patient was taken off the study. Two other patients had dose-reduction and the median days of platelet nadir was 42.

In all the other patients, the delay in restarting a subsequent cycle was never greater than 2 weeks and dose reduction was not required unless grade 3–4 toxicity occurred.

Reported grade 3/4 non-haematological adverse events that seemed to be related to drug administration were vomiting (NHPT n = 1, HPT n = 2), abdominal pain (HPT n = 1) and anorexia (NHPT n = 2). No patient developed fever and neutropaenia. Four patients, 2 in the NHPT and 2 in the HPT groups, developed herpetic infections requiring intravenous acyclovir (2 herpes zoster and 2 varicella). Three of these 4 patients had associated severe lymphopenia, including 1 with a severe depletion of CD4$^+$ lymphocytes. None of those patients were on steroids. Other toxicities were mild and included fatigue, fever, flu-like symptoms, gastrointestinal symptoms, lethargy, decreased motor function and restlessness. A non-specific grade II rash was also described in 6 patients.

3.3. *Pharmacokinetics*

The pharmacokinetic parameters for 19 patients (9 in the HPT group and 10 in the NHPT group) are shown in Table 5. For each dose level, there were 4–5 patients evaluable, except for the dose level of 65 mg/m^2 in the HPT group, where only 1 patient was studied. Peak concentration and the area under the curve (AUC) increased with increasing doses (Fig. 1). The

Table 5 – Temozolomide (TMZ) pharmacokinetics summary (mean ± standard deviation)

Dose (mg/m^2)	n	Stratum	t_{max} (h)	C_{max} (mg/l)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg/l · h)	Cl/F (l/h/m^2)	V_d/F (l/m^2)
50	5	B	1.60 ± 0.42	2.42 ± 0.61	2.74 ± 3.13	10.66 ± 7.70	6.91 ± 4.00	16.46 ± 7.11
65	1	B	1.00	3.57	0.92	9.03	7.29	9.68
75	5	A	0.90 ± 0.22	5.57 ± 1.78	0.94 ± 0.18	13.32 ± 1.37	5.72 ± 0.65	7.78 ± 1.74
85	4	B	1.25 ± 0.65	5.23 ± 1.69	1.42 ± 0.17	15.30 ± 3.65	5.74 ± 1.32	11.95 ± 3.69
100	3	A	2.75 ± 1.50	3.51 ± 1.26	1.93 ± 0.89	13.66 ± 4.64	8.19 ± 3.70	21.99 ± 11.17

AUC, area under the curve; HPT, heavily pre-treated; NHPT, not heavily pre-treated.

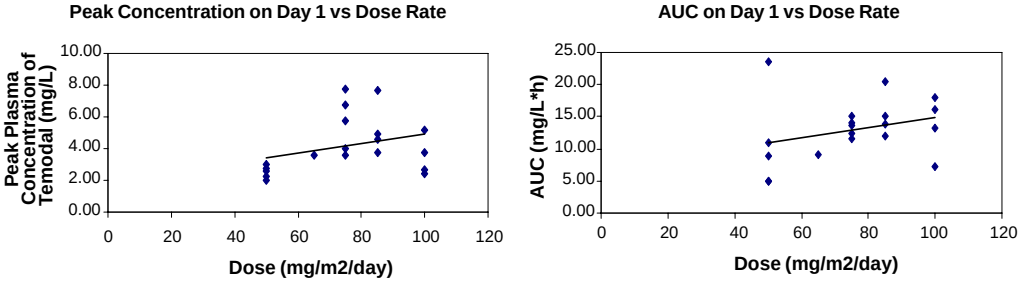

Fig. 1 – C_{max} and area under the curve (AUC) related to dose.

data demonstrate linear pharmacokinetics, but there is substantial inter-patient variability, as outlined in Table 5. Five out of the 6 patients who had grade 3/4 lymphopenia and thrombocytopenia had pharmacokinetic studies done. There were no obvious differences between their pharmacokinetic values and those of the patients treated at the same dose levels without serious myelotoxicity.

With such a limited sample size in each cohort and various dose levels, it was not possible to identify a drug interaction that would be responsible for these pharmacokinetic variations, although a drug interaction seems unlikely due to the fact that the conversion of TMZ to its metabolite 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) is non-enzymatic.

3.4. *Anti-tumour activity*

Over the entire treatment period, 4 of 27 patients (15%) had a response (2 CR and 2 PR). The 2 CR were observed after 2 and 3 cycles, respectively, while the 2 PR were seen after 1 cycle. The response durations of the 4 responding patients were 24, 45, 58 and 90 weeks, respectively, with a median of 74.5 weeks. The 2 patients who showed a CR had a PNET/medulloblastoma and the 2 patients with a PR had a high-grade glioma. Stable disease was observed in 9 patients (4 NHPT and 5 HPT), 5 of them having a diagnosis of low-grade glioma. One patient with refractory ependymoma was classified as SD and had a complete surgical resection of his tumour following 6 cycles of TMZ and received an additional 6 cycles and is still alive in CR at the last follow-up.

The median overall survival (OS) time was 20.6 weeks (95% CI 10.9–160.6) and the 1-year OS rate was 40.1% (95% CI 25.3–63.7). Twenty-three patients (85.2%) experienced disease progression while on study, with a median progression-free time of 7.6 weeks (95% CI 5.9–45.3) and a 1-year progression free survival (PFS) rate of 22.2% (95% CI 11.0–45.0). No statistically significant difference was found in either OS ($P = 0.92$) or PFS ($P = 0.64$) between HPT and NHPT patients.

4. Discussion

TMZ has demonstrated activity and safety in the treatment of brain tumours. Several phase I and II studies have been conducted using TMZ given over a 5-d course administered every 4 weeks in adults[11,13,14,34,35] as well as in children.[11,21,22] These studies have demonstrated the safety of TMZ and have suggested some activity in glioblastoma, anaplastic astrocytomas and low-grade gliomas. Recently, Stupp and colleagues reported a statistically significant and clinically meaningful survival benefit in adult patients with glioblastoma. This was a randomised study comparing radiotherapy alone or radiotherapy plus metronomic TMZ given daily during radiation, followed by a non-metronomic maintenance regimen that used the classical TMZ 5 d regimen every 28 d. Results were very encouraging, the median survival being 14.6 months with radiotherapy plus TMZ and 12.1 months with radiotherapy alone, the 2-year survival rate being 26.5% with radiotherapy plus TMZ, compared with 10.4% with radiotherapy alone.[37] TMZ studies using the classical 5-d regimen in paediatric brain tumours have, however, been disappointing.[21,22] Extended continuous TMZ administration, or metronomic therapy, has been studied mostly in adults[28,29] although Sterba and colleagues reported 8 paediatric patients with high-risk brain tumours treated with continuous low-dose TMZ and concomitant radiotherapy with encouraging results.[30] Therefore our study represents the first study establishing the safety, pharmacokinetic and dosage of continuous low-dose administration of TMZ. We reported separately a patient with gliomatosis cerebri treated with the same regimen. This patient demonstrated a durable stable disease.[36] The concept of metronomic chemotherapy as a potential anti-angiogenic therapy has been reported in multiple pre-clinical models[23,25,38,39] and appears to be beneficial in some clinical situations.[40–42] Surprisingly, responses to agents provided in low continuous dosing have been documented despite previous progression on conventional doses and schedules of the same agents, including with TMZ.[43–45] Pre-clinical models may provide a possible anti-angiogenic explanation behind tumour response with the metronomic dosing.[24,25] Kurzen and colleagues recently showed that TMZ at low doses inhibits angiogenesis *in vitro* and therefore TMZ anti-tumour activity might be due, at least partially, to anti-angiogenic properties, although the precise mechanism is still unclear.[39]

The purpose of the present study was to demonstrate the feasibility and safety of administering TMZ in an extended schedule to paediatric cancer patients, and to determine its pharmacokinetic profile in children. Our results demonstrate linear pharmacokinetics over a range of doses, but with significant inter-patient variability, as found in previous studies. In this study, pharmacokinetic results in children are consistent with the adult low-dose regimen[2,28] and the published paediatric data from the 5-d schedule.[21,46,47] No correlation was observed between pharmacokinetic data (AUC and peak concentration) and toxicity or response to TMZ. The dose intensity and drug exposure (defined as the cumulative AUC over 28 d) were much higher in the 6-week continuous low-dose schedule, with at least a 1.5-fold increase compared with the 5-d administration schedule (Table 6). As suggested by others,[21,28] the increased drug exposure was not associated with increased toxicity. There were nevertheless some toxicities observed in our study, including prolonged thrombocytopenia and clinically noteworthy grade 3 lymphopenia related to TMZ administration

Table 6 – Comparison of 5-d and 42-d dosing schedule

	Daily × 5	Daily × 42 (HPT/NHPT)	
Daily dose (MTD) (mg/m^2)	200	75/85	
Total dose/course (mg/m^2)	1000	3150/3570	
Dose intensity (mg/m^2/wk)	250	9 weeks*	350/396
		7 weeks*	450/459
Drug exposure (μg · h/ml)	245	9 weeks	572/580
Over a 28-d period		7 weeks	372/386

MTD, maximum tolerated dose; HPT, heavily pre-treated; NHPT, not heavily pre-treated.
* Depending on the rest period.

noted in 9 out of 20 evaluable patients in both groups. Four of those patients had not recovered by the end of the data collection period. Similar occurrence of lymphopenia has been described in an adult study of continuous low-dose TMZ and was associated with an increased risk of opportunistic infections.[48] There was no case of *Pneumocystis carinii* infection in our patients; however, 11 patients were on prophylactic sulphamethoxazole/trimethoprim. There were 4 cases of herpetic infection, including 1 in a patient with severe CD4+ depletion.

We observed responses (CR + PR) in 20% of evaluable patients (with either high-grade glioma or PNET/medulloblastoma). The two CRs were observed in patients with medulloblastoma, including 1 patient who had relapsed post high-dose chemotherapy with stem cell rescue. Stable disease was observed in 9 patients, mostly low-grade gliomas. Seven patients (25%) completed the 6 courses, which is unusual in a phase I/II study. These results are encouraging and in keeping with a previous report of 13 children with progressive low-grade gliomas treated with TMZ where 9 patients received a metronomic dosing over 42 d. Four of these patients had PR and 5 had SD disease. Moreover, the metronomic schedule appeared to be less toxic than the 5-d schedule, especially with regards to myelosuppression.[28,30] Whether the observed increase in drug exposure translates into improved clinical outcome will need to be addressed in future prospective phase II clinical trials.

Metronomic scheduling is associated with increased drug-exposure without an apparent increase in acute toxicity, potentially leading to a better response rate, AGT depletion being the potential biological mechanism. Low-dose continuous scheduling could reduce the emergence of AGT-related drug-resistance as well as potentiate the selective effects of other drugs (i.e. anti-angiogenic agents)[49,50] or radiotherapy on cancer cells. The development and evaluation of a chemotherapeutic regimen using metronomic dosing is promising and needs further mechanistic studies.[51] Establishing surrogate markers of anti-angiogenic activity, such as blood circulating endothelial cells and precursors, will be critical in the future to validate the potential anti-angiogenic activity of such a regimen.[49]

Metronomic administration of low-dose chemotherapy may cause additional new toxicities, such as profound chronic immunosuppression or the emergence of secondary neoplasm. Close monitoring should therefore be instituted to detect such late toxicities. In summary, TMZ given in an extended metronomic oral schedule in children with relapsed brain tumours is well tolerated, with myelosuppression, thrombocytopenia and lymphopenia being the major toxicity. Even though no *Pneumocystis carinii* infections were observed in our study, we would recommend the use of PCP prophylaxis and close monitoring of herpetic infections in all patients treated with this schedule. Encouraging responses were observed and warrant further phase II studies. The recommended dose for future studies is 85 mg/m^2 for this 42-d cycle.

Conflict of interest statement

None declared.

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