European Journal of Neurology 2013, 20: 223–230 doi:10.1111/j.1468-1331.2012.03778.x

REVIEW ARTICLE

The efficacy of temozolomide for recurrent glioblastoma multiforme

Chao Chen[a†], Tao Xu[a†], Yicheng Lu[a], Juxiang Chen[a] and Shenhong Wu[b]

[a]*Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai, China; and* [b]*Division of Hematology and Oncology, Department of Medicine, Stony Brook University School of Medicine, New York, NY, USA*

Keywords:
meta-analysis, recurrent glioblastoma, temozolomide

Received 10 April 2012
Accepted 26 April 2012

Background and purpose: The efficacy of temozolomide (TMZ) in recurrent glioblastoma multiforme (GBM) has been evaluated by several clinical trials. A meta-analysis to assess the overall efficacy of TMZ in the treatment of recurrent GBM was carried out by the authors.

Methods: Medline, EMBASE database and the Cochrane Library were searched for relevant studies. Eligible studies were clinical trials of recurrent GBMs assigned to TMZ with data on efficacy including tumor response, progression-free survival (PFS) or overall survival (OS) available. The overall efficacy was calculated using a random-effects or fixed-effects model, depending on the heterogeneity of the included trials.

Results: A total of 15 phase II clinical trials including 902 recurrent GBMs were analyzed. The overall clinical benefit rate was 50.5% (95% CI: 44.3–56.7%) with significant difference between metronomic and standard schedules of TMZ (61.4% vs. 46.3%, $P = 0.037$). The overall 6-month PFS (PFS-6) rate was found to be 27.8% (95% CI: 22.7–33.5%) with significant difference between metronomic and standard schedules (33.1% vs. 20.1%, $P < 0.001$). In addition, significant difference in PFS-6 was detected between high (average daily dose >100 mg/m^2) and low (average daily dose $\leq$100 mg/m^2) dose metronomic schedules (RR = 1.57, 95% CI: 1.17–2.09, $P = 0.002$). The overall 6-month OS (OS-6) and 12-month OS (OS-12) rates were 65.0% (95% CI: 57.4–71.9%) and 36.4% (95% CI: 26.9–47.1%) separately. There was no significant difference in OS-6 between metronomic and standard schedules ($P = 0.266$); however, a trend was noted favoring the metronomic schedule for OS-12 ($P = 0.089$).

Conclusions: Temozolomide is effective for recurrent GBMs, and its efficacy may be increased with metronomic schedule and high average daily dose (>100 mg/m^2).

Introduction

Glioblastoma multiforme (GBM) is the most frequent malignant brain tumors seen in adults and usually has an extremely poor prognosis. Despite multimodality therapy including maximal resection and adjuvant radio therapy (RT) concurrent with temozolomide (TMZ), followed by 6 months of adjuvant TMZ, the overall outcome of patients with GBM remains dismal [1], and nearly all GBMs recur. Prognosis for patients with recurrent GBMs is poor and treatment options are limited. Currently there is no consensus on the optimal approach for recurrent disease.

Temozolomide is an alkylating chemotherapeutic agent that is the standard treatment for newly diagnosed GBMs. A phase III trial showed that concomitant RT and TMZ (75 mg/m^2 per day for 6 weeks) plus six cycles of adjuvant TMZ (150–200 mg/m^2 for 5 days every 28-day cycle) improved 2-year survival versus RT alone from 10.4% to 26.5%. At a median follow-up of 28 months, the median survival was 14.6 months with RT plus TMZ and 12.1 months with RT alone [1,2]. A later analysis showed that benefits of adjuvant TMZ with RT lasted throughout the 5 years of follow-up [3].

Temozolomide was first approved for the treatment of recurrent GBM using the standard 5-day

Correspondence: Y. Lu, Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai, China (tel.: +86 21 81885696; fax: +86 21 63586116; e-mail: lyc305@126.com).
J. Chen, Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai, China (tel.: +86 21 81885677; fax: +86 21 63586116; e-mail: juxiangchen.cz@gmail.com).
†The first two authors contributed equally to the work.

EUROPEAN JOURNAL OF NEUROLOGY
THE OFFICIAL JOURNAL OF THE EUROPEAN FEDERATION OF NEUROLOGICAL SOCIETIES

regimen (150–200 mg/m^2 for 5 days every 28-day cycle). The standard regimen of TMZ is based on pre-clinical and phase I clinical studies showing schedule dependency [4,5]. In the randomized phase II study that compared the efficacy of TMZ with procarbazine in GBM patients at first recurrence, TMZ improved 6-month progression-free survival (PFS-6: 21% vs. 8%, $P = 0.008$), median PFS (12.4 weeks vs. 8.3 weeks, $P = 0.006$) and 6-month overall survival (OS-6: 60% vs. 44%, $P = 0.019$) [6]. Several single-arm phase II clinical trials showed that PFS-6 with TMZ ranged between 18.0% and 31.8% in recurrent GBM [7–12].

Temozolomide acts by methylating bases within DNA, which subsequently produces DNA double-strand breaks and induces apoptosis. The DNA damage caused by TMZ is repaired by the cellular repair enzyme O6-methylguanin-DNA-methltransferase (MGMT), which reverts its activity by removing cytotoxic methyl adducts from the DNA [13]. Several studies have suggested that resistance to TMZ is primarily mediated by MGMT [13,14]. Because MGMT is irreversibly inactivated during DNA repair process, the enzyme needs to be continuously refueled by de novo protein synthesis. Therefore, metronomic schedule of TMZ may lead to MGMT depletion and overcome the inherent resistance of glioma cells. 'Metronomic' schedule is defined as frequent administration of certain cytotoxic agents at low doses compared with conventional chemotherapy [15]. Furthermore, it has been demonstrated that TMZ decreases MGMT activity in peripheral mononuclear cells in a schedule-dependent manner [16], and protracted administration of TMZ results in more extensive and sustained depletion of MGMT [17].

Based on the observations, several clinical trials tried to evaluate the efficacy of metronomic schedule of TMZ in recurrent GBMs [18–25]. However, the objective response (OR) rates varied widely (0–30.9%) as well as the PFS-6 (19.0–48.0%). It is possible that the variation may result from different metronomic schedules used for these trials or limited number of patients in each trial.

To improve the outcome of TMZ treatment, a systematic review and meta-analysis was performed by the authors to determine the overall efficacy of TMZ and to understand underlying causes of the variation.

Methods

Data source

The literature search was conducted in the Medline (via PubMed), EMBASE database and the Cochrane Library (until Nov, 2011). Our search strategy included the terms 'temozolomide', 'temodar', 'temodal', 'brain tumor', 'brain neoplasms', 'glioma' and 'glioblastoma' and was restricted to human clinical trials published in English. Additionally, we manually searched the reference lists of all accepted papers to ensure that no studies were missed. The following search strategy was used to search Medline: ((('Brain Neoplasms' [Mesh]) OR ('Glioblastoma' [Mesh]) OR (brain tumo?r*) OR ('Glioma' [Mesh])) AND ((temozolomide) OR (temodar) OR (temodal)) AND (Humans [Mesh] AND Clinical Trial [ptyp] AND English [lang]).

Selection criteria and process

Studies that met the following criteria were chosen for analysis: (i) prospective clinical trials designed to evaluate the efficacy of TMZ in recurrent GBMs; (ii) patients assigned to treatment with TMZ as a single agent at recurrence; (iii) the eligible patients enrolled should be adult patients ($\geq$18 years) with Karnofsky Performance Status score $\geq$60 and normal hematologic, renal, and hepatic function; (iv) GBM patients enrolled no <20; (v) data available for tumor response, PFS or OS. All the potentially relevant papers were reviewed independently by two investigators (CC and TX) and disagreements were resolved by discussion and consensus. Details on trial design, patient characteristics, TMZ dose and schedule, tumor response rates and follow-up of progression and survival were also extracted independently by the two investigators. When progression or survival data were solely provided in graphical form, figures were digitized to extract the numerical values using Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/).

Clinical endpoints

Clinical endpoints included tumor response rates, PFS-6, OS-6 and OS-12. PFS-6 was the primary endpoint, as it is widely accepted and mostly recorded. Tumor response was recorded according to MacDonald's criteria as follows: [26] complete response (CR) – disappearance of all radiographically measurable lesions and no evidence of new lesions; partial response (PR) – a $\geq$50% but <100% reduction in the enhancing component of all brain lesions with no new lesions; progressive disease (PD) – a $\geq$25% increase in the enhancing tumor or the appearance of new lesions; stable disease (SD) – all other situations.

Statistical analysis

All statistical analyses were made using Comprehensive Meta-Analysis program version 2 (Biostat, Englewood, NJ, USA). For each meta-analysis, the Cochrane's Q statistic was first calculated to assess the heterogeneity of the included trials. For P-values <0.1, the assumption of homogeneity was deemed invalid [27], and the random-effects model was used. The fixed-effects model was chosen when $P \geq 0.1$. For the analysis of the primary endpoint (PFS-6), substantial efforts were made to explore the potential reasons for the heterogeneity. A two-tailed P-value of <0.05 was deemed statistically significant.

Role of the funding sources

The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had access to all the raw data and had the final responsibility to submit the manuscript for publication.

Results

Identification of relevant studies

The flow diagram of the selection process for relevant studies is shown in Fig. 1. Our search yielded a total of 360 articles. Three-hundred and twenty-five articles were excluded for not being related to the topic after reviewing the titles and abstracts, and the remaining 35 articles were reviewed further [6–12,18–25,28–47]. Twenty studies were considered to be ineligible for inclusion for the following reasons: (i) eight studies enrolled no GBM patients or the data about GBMs cannot be extracted [30,34,37–39,43,45,46]; (ii) eight studies enrolled <20 GBM patients [29,31,35,36,40–42,47]; (iii) two studies did not describe the clinical endpoints of interest [32,33]; (iv) one study took the MRC scale to evaluate the treatment response [28]; (v) one study mixed the efficacy data of TMZ with another alkylating agent carmustine [44]. Finally, 15 clinical trials were included in the meta-analysis. It should be noted that the trial conducted by Perry *et al.* [25] adopted the 'Response Evaluation Criteria in Solid Tumor' for treatment response, so the data about tumor response were not included in analysis, but the progression and survival data were still used as they were not affected.

Characteristics of the included studies

The 15 clinical trials included for analysis (Table 1) were comprised of 14 single-arm phase II clinical trials [7–12,18–25] and one randomized phase II clinical trial that compared TMZ versus procarbazine as the reference arm [6]. Seven of the 15 trials used the standard 5-day regimen, a dose of 150–200 mg/m^2 for five

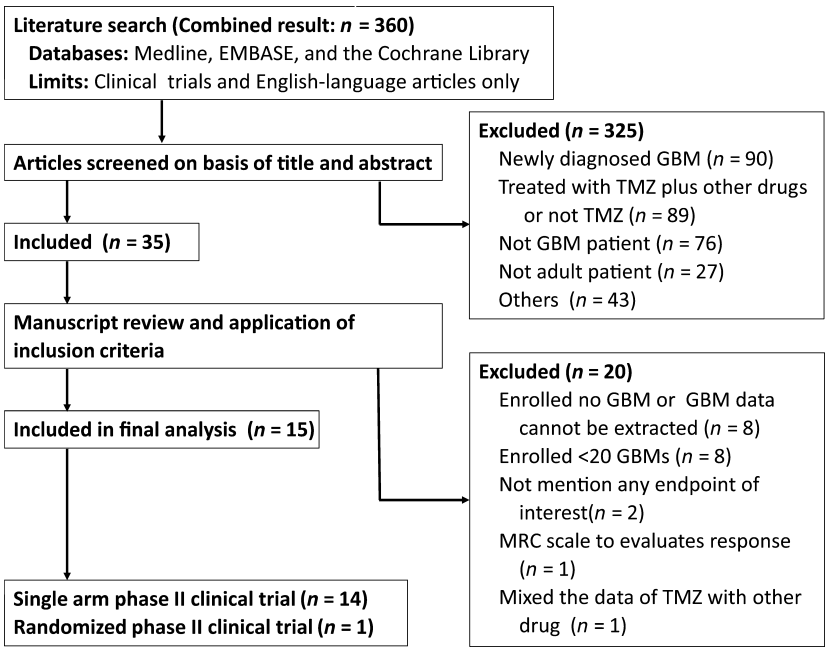

Figure 1 Clinical studies identified and screened for eligibility.

Table 1 Characteristics and efficacy results of clinical trials included in the meta-analysis

Type[a]	Reference	Country	Trial design	Schedule	Patients enrolled	Prior[b] TMZ	First relapse	Tumor response			PFS-6 (%)	OS-6 (%)	OS-12 (%)
								CR	PR	SD			
Standard	Yung *et al.* [6]	Canada	Randomized Phase II (TMZ versus Procarbazine)	150–200 mg/m^2 days1–5 q 28	112	No	Yes	0	6	45	21.0	60.0	22.8[c]
	Brada *et al.* [7]	United States	Single-arm phase II	150–200 mg/m^2 days1–5 q 28	128	No	Yes	2	8	57	18.0	46.0	14.6[c]
	Brandes *et al.* [8]	Italy	Single-arm phase II	150–200 mg/m^2 days1–5 q 28	21	No	No	2 (20)[d]	3 (20)[d]	4 (20)[d]	31.8	68.2	NA
	Harris *et al.* [9]	Australia	Single-arm phase II	150–200 mg/m^2 days1–5 q 28	25	NA	Not All	0	5	6	NA	NA	NA
	Brandes *et al.* [10]	Italy	Single-arm phase II	150–200 mg/m^2 days1–5 q 28	42	No	No	2	6	9	24.	64.0	28.0
	Chang *et al.* [11]	United States	Single-arm phase II	200 mg/m^2 days1–5 q 28	142	No	Not All	1	21	43	18.0	60.0	21.3[c]
	Hassler *et al.* [12]	Australia	Single-arm phase II	150–200 mg/m^2 days1–5 q 28	30	Not All	Yes	2	5	4	NA	84.0[c]	65.0[c]
Metronomic Low dose	Khan *et al.* [18]	United States	Single-arm phase II	75 mg/m^2 days1–42 q 72	28	No	NA	0	0	11	19.0	60.0	32.1[c]
	Brandes *et al.* [20]	Italy	Single-arm phase II	75 mg/m^2 days1–21 q 28	33	No	Yes	1	2	17	30.3	73.0	38.0
	Kong *et al.* [24]	South Korea	Single-arm phase II	40–50 mg/m^2 continuous	38	Yes	Yes	0	2	21	32.5	56.0	47.6[c]
	Perry *et al.* [25]	Canada	Single-arm phase II	50 mg/m^2 continuous	120	Yes	Yes	NA	NA	NA	23.9	NA	23.7
High Dose	Wick *et al.* [19]	German	Single-arm phase II	150 mg/m^2 days1–7, 15–21 q 28	21	No	Not All	0	2	17	48.0	NA	81.0
	Caroli *et al.* [21]	Italy	Single-arm phase II	150 mg/m^2 days1–5, 75 mg/m^2 days 6–10 q 28	30	No	Yes	NA	NA	NA	36.6	93.3[c]	63.3
	Wick *et al.* [22]	German	Single-arm phase II	150 mg/m^2 days1–7, 15–21 q 28	64	Not All	Not All	1 (45)[d]	6 (45)[d]	NA	43.8	NA	NA
	Balmaceda *et al.* [23]	United States	Single-arm phase II	200 mg/m^2 loading dose[e] 9 dose at 90 mg/m^2 q 12 h	68	No	Not All	3	18	22	35.0	71.0	35.0

CR, complete response; PR, partial response; SD, stable disease; PFS-6, 6-month progression-free survival; OS-6, 6-month overall survival; OS-12, 12-month overall survival; NA, not available; TMZ, temozolomide.

[a]All included trials are divided into Standard (standard 5-day schedule) schedule group and Metronomic schedule group; Metronomic schedule group is further divided into two subgroups: Low Dose (metronomic schedule with average daily dose $\leq$100 mg/m^2) and High Dose (metronomic schedule with average daily dose >100 mg/m^2).

[b]Whether treated with temozolomide before.

[c]Data extracted using Engauge Digitizer version 4.1.

[d]Figure in round brackets represents the number of patients eligible for tumor response evaluation.

[e]200 mg/m^2 loading dose at first day followed by 9 consecutive dose at 90 mg/m^2 every 12 h.

consecutive days every 28-day cycle [6–12]. The remaining eight trials used metronomic schedules, and they were further divided into two subgroups based on average daily dose: low dose metronomic (metronomic schedule with average daily dose $\leq$100 mg/m^2) [18,20,24,25], and high dose metronomic (metronomic schedule with average daily dose >100 mg/m^2) [19,21–23]. Details of doses are shown in Table 1. It should be noted that the trial conducted by Balmaceda *et al*. [23] was assigned to the 'high dose metronomic' group with a schedule of 200 mg/m^2 loading dose on the first day followed by nine consecutive doses at 90 mg/m^2 every 12 h. Although the schedule is not obviously continuous, it still reflects the idea of frequent low dose chemotherapy. A total of 902 patients were available for analysis, of whom 500 patients for standard schedule and 402 patients for metronomic schedule. Trials were conducted in the United States, Canada, Italy, Germany, Australia and South Korea. Five of the 15 included trials were supported by research grants from the pharmaceutical industry [7,11,18,23,24].

Tumor response

Both OR (CR and PR) rate and clinical benefit (CR, PR and SD) rate were analyzed. As very few patients can achieve CR, the data of CR were not analyzed separately. Data for OR were available for analysis from a total of 13 trials including 732 patients. The OR rate ranged from 0% to 30.9%, with the lowest noted in a low dose metronomic schedule [18], and the highest noted in a high dose metronomic schedule [23]. The overall rate of OR was 14.0% (95% CI: 9.8–19.7%) as determined by the random-effects model (heterogeneity analysis: $Q = 35.313$, $I^2 = 66.018$, $P < 0.001$). For clinical benefit rate, data of 687 patients enrolled in 12 trials were available for analysis. The clinical benefit rate ranged from 36.7% to 90.5%, with the lowest noted in a standard 5-day schedule [12], and the highest again noted in a high dose metronomic schedule [19]. The overall rate of clinical benefit was 50.5% (95% CI: 44.3–56.7%) as determined by the random-effects model (heterogeneity analysis: $Q = 24.521$, $I^2 = 55.151$, $P = 0.011$, Fig. 2a).

To explore the heterogeneity of the included studies, tumor response according to TMZ schedules was further analyzed. As shown in Table 2, the metronomic schedule was associated with a clinical benefit rate significantly higher than the standard schedule (61.4% vs. 46.3%, $P = 0.037$), whereas no significant difference in OR was found (11.9% vs. 14.5%, $P = 0.646$).

PFS

Six-month progression-free survival (PFS-6) was the primary clinical endpoint of interest in this study. Data were available for analysis from a total of 847 patients enrolled in 13 trials. The PFS-6 rate ranged between 18.0% and 48.0%, with the lowest noted in two trials using the standard schedule [7,11], and the highest in a trial using the high dose metronomic schedule [19]. Meta-analysis showed that there was a heterogeneity in PFS-6 rates for the included studies ($Q = 32.153$, $I^2 = 62.678$, $P < 0.001$), and the overall PFS-6 rate was 27.8% (95% CI: 22.7–33.5%) as determined by the random-effects model (Fig. 2b).

Further analysis to explore the heterogeneity detected a significant difference in PFS-6 between the metronomic schedule and standard schedule (33.1% vs. 20.1%, $P < 0.001$, Table 2). In addition, the high dose metronomic schedule achieved PFS-6 rate significant higher than the low dose (RR = 1.57, 95% CI: 1.17–2.09, $P = 0.002$).

Additionally, we examined other underlying causes including prior TMZ treatment or whether it is first relapse for heterogeneity. However, the results indicated that PFS-6 rate did not vary significantly with prior TMZ treatment ($P = 0.729$) or first relapse ($P = 0.322$).

OS

Overall survival rates at 6-month (OS-6) and 12-month (OS-12) were analyzed separately. Data for OS-6 were available for analysis from a total of 672 patients enrolled in 11 trials. The OS-6 rate ranged from 46.0% to 93.3%, with the lowest noted in a trial using the standard schedule [7], and the highest noted in a trial using the high dose metronomic schedule [21]. The overall OS-6 rate was 65.0% (95% CI: 57.4–71.9%) determined by random-effects model (heterogeneity analysis: $Q = 32.461$, $I^2 = 69.193$, $P < 0.001$). For OS-12, data of 792 patients enrolled in 12 trials were available for analysis. The OS-12 rate ranged from 14.6% to 81.0%, with the lowest noted in a trial using the standard schedule [7] and the highest again noted in a trial using the high dose metronomic schedule [19]. The overall OS-12 rate was 36.4% (95% CI: 26.9–47.1%) as determined by the random-effects model (heterogeneity analysis: $Q = 76.021$, $I^2 = 85.53$, $P < 0.001$, Fig. 2c).

Further analysis did not detect significant difference in OS-6 rate between the metronomic and standard schedules (69.9% vs. 61.4%, $P = 0.266$, Table 2); however, a trend was noted favoring the metronomic schedule for OS-12 (43.9% vs. 27.4%, $P = 0.089$, Table 2).

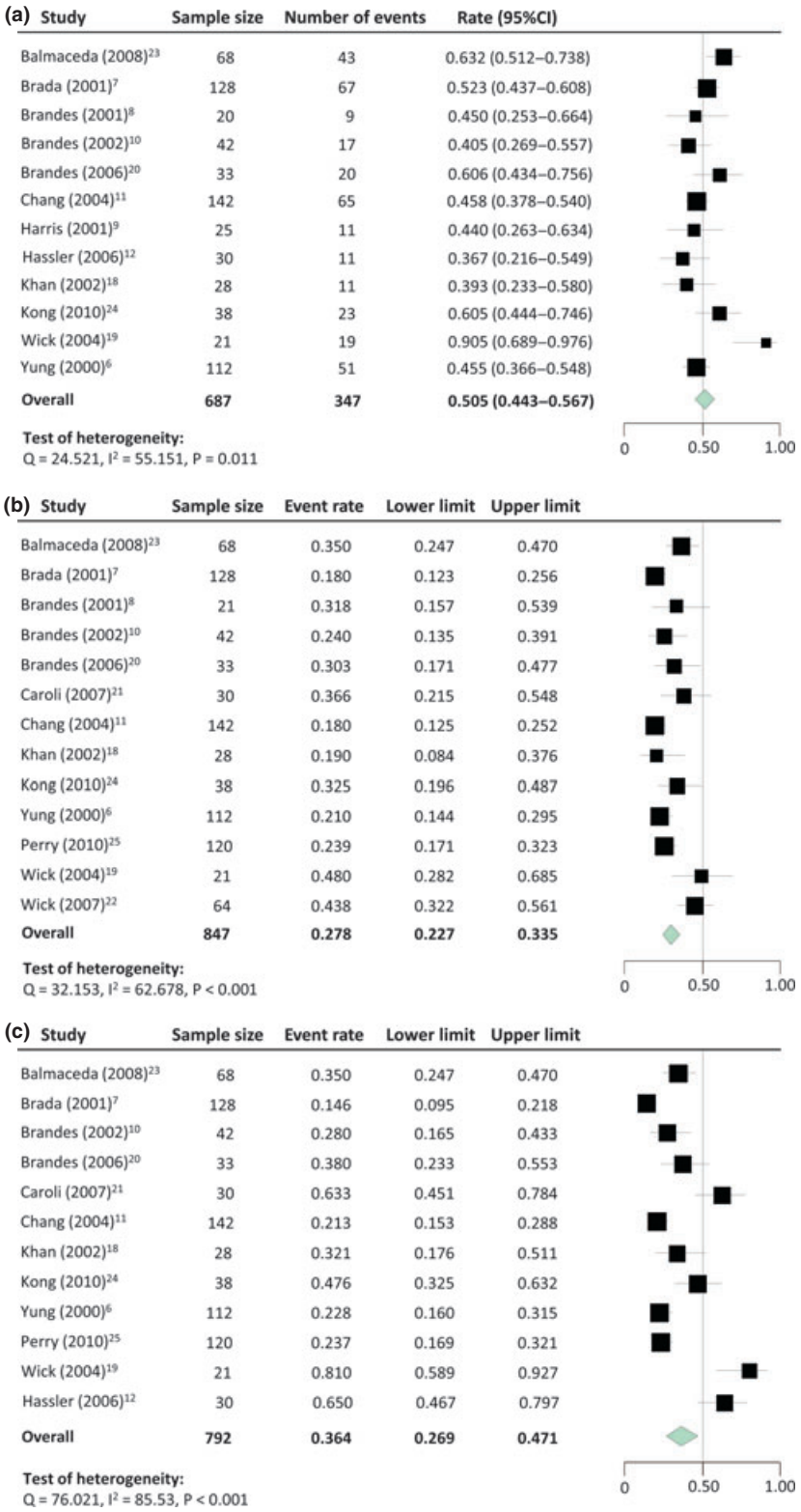

Figure 2 Efficacy of TMZ for recurrent GBM including clinical benefit (a), 6-month PFS (b), 12-month OS (c). Summary rates were calculated by meta-analyses using the random effect model. Clinical benefit: CR + PR + SD; PFS-6: 6-month progression-free survival; OS-12: 12-month overall survival. Size of squares is directly proportional to amount of information available.

Table 2 Comparison of various clinical endpoints between the metronomic and standard schedules of TMZ

	OR[a]		Clinical benefit[b]		PFS-6		OS-6		OS-12	
	n	Rate (%)	*n*	Rate (%)	*n*	Rate (%)	*n*	Rate (%)	*n*	Rate (%)
Overall	732	14.0 (9.8–19.7)	687	50.5 (44.3–56.7)	847	27.8 (22.7–33.5)	672	65.0 (57.4–71.9)	792	36.4 (26.9–47.1)
Standard	499	14.5 (9.6–21.3)	499	46.3 (42.0–50.7)	445	20.1 (16.6–24.1)	475	61.4 (52.2–69.9)	454	27.4 (16.8–41.4)
Metronomic	233	11.9 (5.6–23.7)	188	61.4 (47.9–73.3)	402	33.1 (26.9–40.0)	197	69.9 (57.3–80.0)	338	43.9 (30.8–57.9)
P-value*		$P = 0.646$*		**$P = 0.037$***		**$P < 0.001$***		$P = 0.266$*		$P = 0.089$*

[a]'OR' includes CR and PR.

[b]'Clinical Benefit' includes CR, PR and SD.

*Bold values indicate a statistically significant difference between metronomic and standard schedules ($P < 0.05$).

Discussion

Temozolomide is a new alkylating agent whose efficacy in recurrent GBMs is not well-defined. This study showed that PFS-6 and OS-12 rates for recurrent GBM patients treated with TMZ were 27.8% (95% CI: 22.7–33.5%) and 36.4% (95% CI: 26.9–47.1%), respectively. In addition, the overall clinical benefit rate was 50.5% (95% CI: 44.3–56.7%) with an OR (CR and PR) rate of 14.0% (95% CI: 9.8–19.7%). These results were superior to those of cytostatic and cytotoxic agents for the treatment of recurrent glioma patients as analyzed by another meta-analysis of eight consecutive phase II trials, in which the PFS-6 rate was 15% for recurrent GBMs, with an OR rate of 6% and clinical benefit rate of 33% [48]. This is further supported by the only randomized controlled trial (RCT) included in this study, which was also in favor of TMZ compared to procarbazine with respect to PFS-6 (HR = 1.54, $P = 0.008$) [6].

In this study, we showed that the metronomic schedule of TMZ may be significantly superior to the standard 5-day regimen with respect to PFS-6 ($P < 0.001$) and clinical benefit rate ($P = 0.037$). In addition, there was a trend favoring the metronomic schedule for OS-12 rate ($P = 0.089$). This may be attributed to reduced development of TMZ resistance or increased antiangiogenesis associated with the metronomic schedule. MGMT is thought to be responsible for TMZ resistance, and it has been demonstrated that TMZ decreases MGMT activity in a schedule-dependent manner, with protracted administration of TMZ resulting in more extensive and continuous depletion of MGMT [16,17]. In addition to suppression of MGMT activity, experiment *in vitro* has indicated that low dose TMZ at a concentration equivalent to 20 mg/m^2 every 8 h inhibits angiogenesis [49]. This is because conventional chemotherapy, which is administered at more toxic 'maximum tolerated doses', requires 2–3 weeks' breaks between successive cycles of therapy. The long interval between cycles permits the survival and regrowth of a fraction of vascular endothelial cells, allowing tumor angiogenesis to persist and tumor growth to resume. Continuous or near continuous, small-dose chemotherapy, on the other hand, enhances the antiangiogenic and proapoptotic effects of chemotherapy agents on both tumor cells and endothelial cells [15,50]. These results are consistent with the findings that GBMs are among the most vascularized of tumors in humans [51], and several angiogenesis inhibitors, such as bevacizumab, have shown efficacy in clinical trials [52]. In the meta-analysis by Wong *et al.* [53], the PFS-6 and OS-6 rates of recurrent GBMs treated with bevacizumab were 45% (95% CI: 34–57%) and 76% (95% CI: 69–84%), respectively.

Our results showed that the low dose metronomic schedule may be significantly inferior to the high dose metronomic schedule in PFS-6 (RR = 1.57, 95% CI: 1.17–2.09, $P = 0.002$), suggesting a dose-dependent effect. In a randomized phase II trials conducted by Brada *et al.* [46] enrolling 447 recurrent high-grade glioma patients, TMZ-5 (standard 5-day regimen) was found superior to TMZ-21 (100 mg/m^2 for 21 days per 28-day cycle) with respect to overall PFS and showed a 2-month increase in median survival. This trial and our study both indicated that the metronomic schedule of TMZ with a low daily dose ($\leq$100 mg/m^2) may not be optimal for the treatment of GBM in comparison with the high dose schedule. In pharmacokinetic terms, it may be the real drug exposure achieved with a higher daily dose, rather than higher area under the curve, that is the principal determinant of cytotoxicity [46]. The dose–efficacy relationship will need to be determined by further studies. Currently, a phase II clinical trial comparing the safety and efficacy of high and low metronomic schedules (120 mg/m^2, d1–7, 14–21 q 28 days vs. 80 mg/m^2, d1–21 q 28 days) in recurrent GBM is ongoing [54].

Prior TMZ treatment may affect MGMT status of tumor and cause more resistance to TMZ compared to those without prior TMZ exposure. However, our results showed that it was not a source of heterogene-

ity in the included trials ($P = 0.729$). It is possible that other chemotherapeutic drugs applied to GBMs, such as BCNU, CCNU, may induce a resistance mechanism similar to TMZ [55]. Our results also indicated that PFS-6 rate did not vary significantly with the time of relapse ($P = 0.322$). As this analysis involved limited trials, the result should be accepted with caution. Alternatively, these results may be limited by small sample sizes.

Toxicities related to TMZ treatment were not analyzed in this study because of the very limited data availability. Generally, TMZ treatment was well tolerated in both standard and metronomic schedules. The most common hematologic toxicities included lymphopenia, thrombocytopenia, and the most common non-hematologic toxicities included nausea, vomiting and elevation of liver enzyme. The highest rate of grade 3/4 lymphopenia was 24.2% noted in a trial using the low dose metronomic schedule [20], and the highest rate of grade 3/4 thrombocytopenia was 10% noted in a trial using the standard schedule [7]. The rate of grade 3/4 nausea and vomiting was about 5%, with the elevation of liver enzyme <3%.

This study has several limitations. As with any meta-analysis, the findings described here are affected by the limitations of individual clinical trials included in the analysis. All trials involved are phase II clinical trials, and almost all are single-arm trials except the study conducted by Yung *et al.* [6] that compared the efficacy of TMZ with procarbazine. To make the baseline characteristic of included patients comparable, we restricted to adult recurrent GBM patients ($\geq$18 years) with Karnofsky Performance Status score $\geq$60 and normal hematologic, renal and hepatic function in our analysis. Furthermore, significant heterogeneity exists in these studies enrolled. Substantial efforts were made to explore the possible causes for heterogeneity and found that different schedules or average daily dose could explain the heterogeneity adequately. Random-effects model was used when heterogeneity exists within a group to minimize the bias. Additionally, a bias may result from exclusion of certain trials. As two trials including both GBMs and other grade gliomas (mostly grade 3 gliomas) did not provide the data of GBMs [45,46], they were excluded in the analysis. Finally, even though we detected significant difference between the metronomic ($n = 402$) and standard ($n = 445$) schedules and also between high ($n = 183$) and low ($n = 219$) dose metronomic schedules in PFS-6 rate, this finding might be limited by other potential confounding factors.

In conclusion, our study has provided a comprehensive evaluation of TMZ as a treatment of recurrent GBM. Currently, the best available evidence on the efficacy of TMZ is derived from several single-arm phase II clinical trials. Our meta-analysis of these trials has demonstrated that TMZ is effective in this setting with an overall PFS-6 rate of 27.8% (95% CI: 22.7–33.5%) and clinical benefit rate of 50.5% (95% CI: 44.3–56.7%). Furthermore, it has been shown that the metronomic schedule of TMZ may achieve significantly higher efficacy than the standard schedule in PFS-6 and clinical benefit and have a favoring trend in OS-12. Conducting RCTs to evaluate the efficacy of TMZ in the setting of recurrent GBM with the ultimate goal to establish the best therapeutic approach is strongly recommended.

Acknowledgements

This work was supported by National Natural Science Foundation (No. 30930094, 81101907, 30973076).

Reference

A full list of references is available at wileyonlinelibrary.com/journal/ene; doi:10.1111/j.1468-1331.2012.03778.x