Clinical Oncology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Oncology

journal homepage: www.clinicaloncologyonline.net

Original Article

Metronomic Temozolomide in Heavily Pretreated Patients With Recurrent Isocitrate Dehydrogenase Wild-type Glioblastoma: A Large Real-Life Mono-Institutional Study

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Abstract

Aims: Glioblastoma (GBM) is the most common primary malignant brain tumour in adults and frequently relapses. The aim of this study was to assess the efficacy and safety of metronomic temozolomide (TMZ) in the recurrent GBM population.
Materials and methods: All patients treated at our centre between September 2013 and March 2021 were retrospectively reviewed. The main inclusion criteria were first-line therapy with the Stupp protocol, relapse after the first or subsequent line of therapy, treatment with a metronomic TMZ schedule (50 mg/m^2 continuously) and histological diagnosis of isocitrate dehydrogenase wild-type GBM according to World Health Organization 2016 classification.
Results: In total, 120 patients were enrolled. The median follow-up was 15.6 months, the median age was 59 years, Eastern Cooperative Oncology Group performance status (ECOG-PS) was 0–2 in 107 patients (89%). O^6-methylguanine-DNA-methyltransferase (MGMT) was methylated in 66 of 105 (62%) evaluable patients. The median number of prior lines of treatment was 2 (range 1–7). Three (2%) patients showed a partial response; 48 (40%) had stable disease; 69 (57%) had progressive disease. The median overall survival from the start of metronomic TMZ was 5.4 months (95% confidence interval 4.3–6.4), whereas the median progression-free survival (PFS) was 2.6 months (95% confidence interval 2.3–2.8). At univariate analysis, MGMT methylated and unmethylated patients had a median PFS of 2.9 and 2.1 months ($P = 0.001$) and a median overall survival of 5.6 and 4.4 months ($P = 0.03$), respectively. At multivariate analysis, the absence of MGMT methylation (hazard ratio = 2.3, 95% confidence interval 1.3–3.9, $P = 0.004$) and ECOG-PS ≤ 2 (hazard ratio = 0.5, 95% confidence interval 0.3–0.9, $P = 0.017$) remained significantly associated with PFS, whereas ECOG-PS ≤ 2 (hazard ratio = 0.4, 95% confidence interval 0.3–07, $P = 0.001$) was the only factor associated with overall survival. The most common grade 3–4 toxicities were haematological (lymphopenia 10%, thrombocytopenia 3%).
Conclusions: Rechallenge with metronomic TMZ is a well-tolerated option for recurrent GBM, even in pretreated patients. Patients with methylated MGMT disease and good ECOG-PS seem to benefit the most from this treatment.

Key words: Chemotherapy; glioblastoma; IDH; metronomic temozolomide

Introduction

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumour in adults. The median age at diagnosis is 65 years, with an incidence of three to five cases per 100 000 per year. The standard of care for newly diagnosed GBM includes maximal safe resection when

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https://doi.org/10.1016/j.clon.2023.01.012

Please cite this article as: Bosio A et al., Metronomic Temozolomide in Heavily Pretreated Patients With Recurrent Isocitrate Dehydrogenase Wild-type Glioblastoma: A Large Real-Life Mono-Institutional Study, Clinical Oncology, https://doi.org/10.1016/j.clon.2023.01.012

feasible, followed by concomitant chemoradiotherapy and adjuvant temozolomide (TMZ) according to the Stupp scheme [1]. The median overall survival is 12–18 months and the 5-year survival is about 3–5% [2–4].

Because of the disease's aggressive biological behaviour, virtually every GBM patient relapses after first-line therapy, with a median progression-free survival (PFS) and overall survival of 1–6 months and 2–9 months, respectively [3]. Important favourable prognostic factors are younger age, a good performance status and O^6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation [5]. In addition, MGMT promoter methylation is also an important predictor of response to alkylating agents such as TMZ in GBM patients [3]; indeed, MGMT is a repair enzyme that removes methyl or alkyl adducts, representing the principal cause of resistance to TMZ [6].

The standard of care at the time of progression is less well-defined and there is no consensus on the optimal approach for patients with recurrent GBM: second surgery and repeated radiotherapy in selected patients and chemotherapy with nitrosoureas and TMZ rechallenge are the treatment options. However, during the last few years, a greater understanding of the molecular characteristics of GBM has led to the development of new therapeutic strategies, such as the use of small molecules and tyrosine kinase inhibitors [7–14], which are showing promising results.

At relapse, various alternative TMZ schedules have been investigated (i.e. 75 mg/m^2 for 42 of 70 days [14], 75 mg/m^2 for 21 of 28 days [15], 100 mg/m^2 for 21 of 28 days [16], 120 mg/m^2 1 week on/1 week off and 80 mg/m^2 3 weeks on/1 week off [17,18]).

Among them, metronomic TMZ administered continuously at 50 mg/m^2 was analysed in two prospective studies and emerged as a well-tolerated approach in recurrent GBM [19,20]. In addition, preclinical studies suggest that the metronomic schedule may limit endothelial cell recovery, leading to an anti-angiogenic effect [21] and reducing MGMT activity [6].

The aim of this retrospective, mono-institutional study was to evaluate the efficacy and safety of metronomic TMZ rechallenge in a real-life population with recurrent GBM treated at our oncology centre.

Materials and Methods

All patients diagnosed with isocitrate dehydrogenase (IDH) wild-type GBM and treated at our centre were reviewed. Data were collected from electronic medical records at our centre. We retrospectively analysed data stored in a secure database of our neuro-oncology unit. Patients' data were regularly collected and stored into an electronic 'ad hoc database'.

A histologically confirmed diagnosis of IDH wild-type GBM, first-line therapy with concomitant chemoradiotherapy with TMZ and subsequent maintenance therapy with TMZ, relapse after first or subsequent line of therapy and treatment with metronomic TMZ were the main inclusion criteria.

We collected the following molecular features and patients' characteristics: data for the Eastern Cooperative Oncology Group performance status (ECOG-PS) determined at the start of metronomic treatment, as well as data on sex, age at diagnosis, surgery at diagnosis and at recurrence when feasible, the number of cycles of maintenance chemotherapy with TMZ, the number of cycles of metronomic TMZ, the number of lines of treatment prior to metronomic treatment, the time between the last TMZ cycle as first-line therapy and metronomic TMZ administration and the date of death/last follow-up.

Pathological analysis confirmed that all available tissue samples from primary or recurrent tumours represented GBM according to the World Health Organization 2016 classification of tumours of the central nervous system.

The MGMT promoter methylation status was determined by methylation-specific polymerase chain reaction (PCR) or DNA pyrosequencing (cut-off of 7% for methylation); the IDH mutation status was analysed by immunohistochemistry or PCR in the case of patients aged $\leq$55 years.

Treatment consisted of TMZ administered at a dose of 50 mg/m^2/day (i.e. 28 of 28 days), without interruption, until progression or the development of unacceptable haematological or non-haematological adverse effects. One cycle was defined as 28 days.

Response was assessed using magnetic resonance imaging and Response Assessment in Neuro-Oncology (RANO) criteria, whereas toxicity was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE v5.0).

The primary endpoints were overall survival, as determined by the time from the start of the metronomic regimen to the date of death, and factors relating thereto. Secondary endpoints included PFS, as defined from the start of the metronomic TMZ to the date of progression according to RANO criteria, neuroradiological response to treatment (RANO criteria) and safety assessed by using CTCAE v5.0.

For survival analysis, the hazard ratio and 95% confidence interval were calculated by applying the univariate and multivariate Cox regression models. For the execution of the multivariate analysis, the parameters that highlighted a value of $P < 0.2$ for the univariate analysis were taken into account. Survival curves were estimated using the Kaplan–Meier model and the Log-rank test was used to study differences between groups.

Statistical significance was attributed to a value $P < 0.05$ for all analyses. Statistical analysis was carried out using the IBM SPSS software (v.26).

Results

A cohort of 120 patients treated between September 2013 and March 2021 at our centre were enrolled in the study. Patient characteristics are summarised in Table 1. GBM patients included 63 (52.5%) males and 57 (47.5%) females, with a median age at diagnosis of 58 years (range 16–80). The ECOG-PS was 0–2 in 107 patients (89%) and 3

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in 13 patients (11%). Particularly, 95 patients (79.2%) had an ECOG-PS of 0–1, 12 (10%) and 13 (10.8%) had an ECOG-PS of 2 or 3, respectively.

From a molecular perspective, 66 of 105 evaluable patients (62%) presented MGMT promoter methylation. All patients underwent surgery and subsequently received chemoradiotherapy treatment according to the Stupp scheme.

Maintenance chemotherapy with TMZ had been administered to all patients; 62 patients (51.7%) had received fewer than six cycles. The median number of cycles was found to be five (range 1–20). On recurrence, 34 patients (28.3%) underwent second surgery. Among them, nine (26.4%) showed a change in MGMT methylation status over time.

For 70 patients (58.3%), the number of previous lines of therapy (before metronomic TMZ) was less than or equal to two. The remaining 50 patients (41.7%) had received more than two previous lines of treatment. The median number of previous lines of therapy was two (range 1–7). Ninety-four patients (78.3%) had nitrosourea exposure before starting the metronomic treatment. The remaining patients were treated with regorafenib, bevacizumab or carboplatin.

The median time between the last maintenance TMZ cycle and the start of the metronomic schedule was 6 months (range 1–50). In particular, 56 patients (47.5%) received the first metronomic TMZ cycle within 3 months from the end of the last TMZ maintenance cycle.

In our cohort of patients, the median overall survival was 5.4 months (95% confidence interval 4.3–6.4). The 6-month and 12-month overall survival rates were 46.3% (95% confidence interval 37.9–56.5) and 17.1% (95% confidence interval 11.0–26.5). The median PFS was 2.6 months (95% confidence interval 2.3–2.8), whereas the 6-month and 9-month PFS rates were 21.2% (95% confidence interval 14.2–31.5) and 17.2% (95% confidence interval 10.8–27.3) (see Figures 1 and 2).

A univariate analysis analysing the association between individual prognostic factors and the clinical outcome of patients in terms of PFS and overall survival is shown in Table 2.

Statistically significant prognostic factors were the MGMT methylation status and ECOG-PS ≤ 2. Patients with MGMT methylated disease presented a median overall survival of 5.6 months (95% confidence interval 3.8–7.5) versus 4.4 months (95% confidence interval 2.6–6.3) for patients with unmethylated disease ($P = 0.03$) (see Figure 3). The Cox proportional hazard regression model by MGMT subgroups showed a statistically significant association with better overall survival for patients with MGMT promoter methylation (hazard ratio = 0.5, 95% confidence interval 0.3–0.9, $P = 0.017$). The median PFS was 2.9 months (95% confidence interval 2.1–3.6) against 2.1 months (95% confidence interval 1.7–2.5) for patients with non-methylated disease ($P = 0.001$). Patients with an ECOG-PS ≤ 2 at the time of the first metronomic TMZ cycle had a longer overall survival than patients with a poorer PS (ECOG-PS > 2), with a median overall survival of 6 (4.7–7.2) and 2.3 (1.9–2.4) months, respectively ($P < 0.001$).

Table 1
Patient characteristics

	No. patients (%)
Number of patients	120
Gender	
Male	63 (52.5)
Female	57 (47.5)
Median age at diagnosis (years, range)	58 (16–80)
Average age at start of metronomic TMZ (years)	59
ECOG-PS	
Median	2
0–1	95 (79.2)
2	12 (10)
3	13 (10.8)
≤2	107 (89)
>2	13 (11)
MGMT promoter methylation status	
Methylated	66/120 (55); 66/105 (62)*
Unmethylated	39/120 (32.5); 39/105 (38)*
Not available	15 (12.5)
Surgery	
At initial diagnosis	120 (100)
At recurrence	34 (28.3)
First-line therapy	
RT/TMZ	120 (100)
Number of TMZ maintenance cycles	
Median (range)	5 (1–20)
<6	62 (51.7)
≥6	54 (45)
Not available	4 (3.3)
Time TMZ maintenance-metronomic TMZ (months)	
Median (range)	6 (1–50)
≤3	56 (47.5)
>3	49 (41)
Not available	15 (12.5)
Number of prior lines of therapy	
Median (range)	2 (1–7)
≤2	70 (58.3)
>2	50 (41.7)
Median number of metronomic TMZ cycles (range)	2 (1–23)

ECOG-PS, Eastern Cooperative Oncology Group performance status; MGMT, O^6-methylguanine-DNA-methyltransferase; RT, radiotherapy; TMZ, temozolomide.

* One hundred and twenty patients were included in the study, with 105 evaluable for MGMT methylation.

The number of lines of therapy administered prior to metronomic TMZ was not found to be statistically associated with overall survival ($P = 0.23$), nor was the age at the start of therapy ($P = 0.1$), the time in months between the last maintenance TMZ cycle and the first metronomic TMZ cycle ($P = 0.8$) and surgery at recurrence before starting metronomic TMZ ($P = 0.5959$).

At multivariate analysis with the Cox regression model, the absence of MGMT methylation (hazard ratio = 2.3, 95% confidence interval 1.3–3.9, $P = 0.004$) and ECOG-PS (hazard ratio = 0.5, 95% confidence interval 0.3–0.9, $P = 0.017$) were confirmed to be independently associated with PFS in

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a statistically significant manner (Table 3). As for overall survival, the evaluation of ECOG-PS (hazard ratio = 0.4, 95% confidence interval 0.3–0.7, $P = 0.001$) was confirmed to be significantly associated with patients' prognoses, whereas no such role was identified for the MGMT status and the time between the last maintenance TMZ cycle and the first metronomic TMZ cycle (Table 4).

Radiological evaluation of response was carried out on all patients. Three partial responses were observed (2.5%), 48 patients achieved stable disease (40%) and 69 progressed during treatment (57.5%), with a disease control rate and an objective response rate (ORR) of 42.5% and 2.5%, respectively. No complete responses were observed (Table 5).

All patients were evaluable for toxicity. The metronomic schedule was consistently well-tolerated. The most frequent grade 3–4 haematological adverse events were thrombocytopenia (3.3%) and lymphocytopenia (10%). In particular, grade 3 lymphocytopenia was detected in 11 patients (9.2%) and a grade 4 event was observed in one patient (0.8%). Thrombocytopenia was less frequent, with three cases (2.5%) of grade 3 events and one grade 4 event (0.8%). Other grade 3 toxicity events were observed: two cases (1.6%) of leukopenia and one (0.8%) of neutropenia. Non-haematological toxicity was less frequent: two grade 3 hypertransaminasemia events (1.6%), one grade 3 event (0.8%) and one grade 4 fatigue event (0.8%) were reported (Table 6).

Discussion

GBM represents the most aggressive primary brain tumours. Although, in recent years, new therapies have been developed, such as regorafenib, the combination of dabrafenib and trametinib in BRAF V600E-mutated gliomas and neurotrophic tyrosine receptor kinase (NTRK) inhibitors, all of these therapies have shown response in a very limited patient population. In addition, immunotherapy failed to be effective in this setting of patients [22,23].

Combined chemoradiotherapy followed by TMZ maintenance according to the Stupp protocol has continued to be

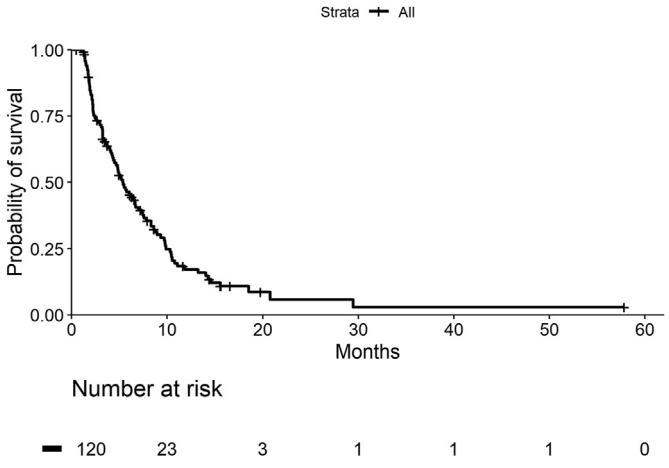

Fig 1. Kaplan–Meier survival curve of median overall survival (5.4 months; 95% confidence interval 4.3–6.4).

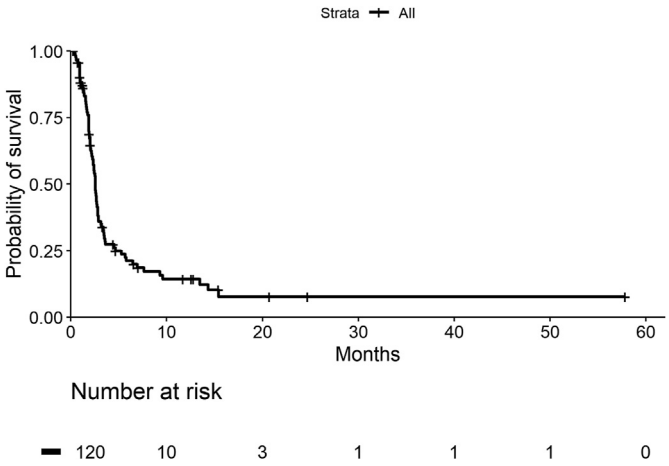

Fig 2. Kaplan–Meier survival curve of median progression-free survival (2.6 months; 95% confidence interval 2.3–2.8).

the standard of care for newly diagnosed GBM patients since 2005 [1]. MGMT promoter methylation is an important predictor of response to alkylating agents such as TMZ, and it occurs in 30–60% of GBM [24].

However, treatment at the time of relapse has not yet been unequivocally defined and treatment options are limited.

TMZ rechallenge with a metronomic schedule can be a treatment option in recurrent GBM patients (see Table 7).

Indeed, the continuous administration of TMZ, which allows the achievement of a higher dose rate than the standard schedule, has proven to be effective in depleting the MGMT enzyme in cancer cells, one of the main factors responsible for resistance to alkylating agents [24]. Particularly, MGMT removes the O^6-alkylguanine DNA adduct through covalent transfer of the alkyl group to its active site and, as a suicide enzyme, it is degraded at the end of each reaction by the ubiquitin-proteasome system. Therefore, extended dose regimens of TMZ can cause a rate of DNA alkylation higher than the MGMT synthesis rate, resulting in enzyme depletion [26].

Moreover, as GBM is a highly vascularised tumour [29–31], this schedule can alter the tumoral angiogenesis.

This real-life study aimed to assess the clinical efficacy and tolerability of rechallenge with metronomic TMZ administered continuously at 50 mg/m^2 to 120 patients with relapsed GBM. The study highlighted a median overall survival of 5.4 months (95% confidence interval 4.3–6.4) and a median PFS of 2.6 months (95% confidence interval 2.3–2.8). A statistically significant advantage in terms of PFS was observed at univariate analysis in patients with methylated MGMT tumours (2.9 months versus. 2.1 with unmethylated MGMT). At multivariate analysis, both the MGMT methylation status (hazard ratio = 2.3, 95% confidence interval 1.3–3.9, $P = 0.004$) and the ECOG-PS confirmed their statistically significant association with PFS (hazard ratio = 0.5, 95% confidence interval 0.3–0.9, $P = 0.01$).

As for overall survival, at univariate analysis, patients with a good ECOG-PS (median overall survival of 6.0

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Table 2
Prognostic impact of clinical-pathological factors on progression-free survival (PFS) and overall survival

		PFS median (months)	*P*-value	Overall survival median (months)	*P*-value
MGMT status			**0.001**		**0.03**
	Methylated	2.9 (2.1–3.5)		5.6 (3.7–7.5)	
	Unmethylated	2.1 (1.7–2.5)		4.4 (2.6–6.3)	
Number of prior lines of therapies			0.06		0.23
	≤2	2.8 (2.5–3.1)		6.0 (4.2–7.8)	
	>2	2.3 (1.8–2.8)		5.1 (3.8–6.3)	
ECOG-PS			0.07		<**0.001**
	≤2	2.6 (2.3–2.8)		6.03 (4.7–7.2)	
	>2	1.8 (0.6–3.06)		2.2 (1.9–2.4)	
Age (years)			0.8		0.8
	<65	2.5 (2.2–2.8)		4.8 (3.2–6.4)	
	≥65	2.8 (2.2–3.3)		6.1 (4.2–7.9)	
Time from standard TMZ to metronomic TMZ (months)			0.26		0.1
	≤3	2.8 (2.2–3.4)		6.7 (1.8–11.5)	
	>3	2.5 (2.3–2.8)		5.3 (4.03–6.6)	

ECOG-PS, Eastern Cooperative Oncology Group performance status; MGMT, O^6-methylguanine-DNA-methyltransferase; TMZ, temozolomide.

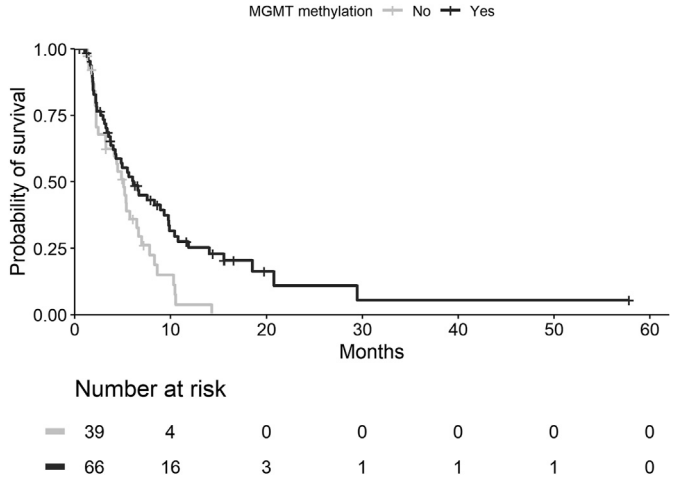

Fig 3. Survival analysis (Kaplan–Meier) based on O^6-methylguanine-DNA-methyltransferase (MGMT) methylation promoter status. The median overall survival was 5.6 months (95% confidence interval 3.8–7.5) and 4.4 months (95% confidence interval 2.6–6.3) for patients with MGMT-methylated and -unmethylated tumour, respectively ($P = 0.03$).

months for ECOG-PS ≤ 2 versus 2.2 months for ECOG-PS > 2) and MGMT methylated status (median overall survival of 5.6 months for methylated diseases versus 4.4 months for unmethylated diseases) reported a longer overall survival. However, at multivariate analysis, only the ECOG-PS at the start of the metronomic schedule was significantly associated with a better overall survival (hazard ratio = 0.4, 95% confidence interval, $P = 0.001$).

The randomised phase II DIRECTOR study by Weller *et al.* [18] evaluated TMZ rechallenge in 105 patients at first progression based on the Stupp scheme. The patients were assigned to two arms: patients in the first arm were treated with 120 mg/m^2 1 week on/1 week off; patients in the second arm were administered 80 mg/m^2 3 weeks on/1 week off. The trial's most important result was the demonstration of a strong prognostic role for MGMT methylation. In this regard, patients with methylated disease had a 6-month PFS of 39.7% and a 1-year overall survival of 54.1% versus 6.9% and 22.9% for patients with non-methylated disease, respectively. Although we found a correlation between MGMT methylation status and patient outcomes, as reported by the DIRECTOR study, this was not confirmed at multivariate analysis. However, it should be noted that our study and the DIRECTOR study present significant differences: not only did we use a different TMZ schedule, but, when available, we considered the MGMT methylation profile at the second surgery (27 of 34 patients who underwent second surgery at relapse). These data are not present in the DIRECTOR study, where the methylation status was only investigated at diagnosis. Indeed, we observed a change in methylation profile of MGMT

Table 3
Multivariate analysis (Cox regression model) for progression-free survival

	Hazard ratio	95% confidence interval	*P*
MGMT status (unmethylated versus methylated)	2.3	(1.3–3.9)	**0.004**
ECOG-PS (≤2 versus >2)	0.5	(0.3–0.9)	**0.01**
Number of prior lines of therapy (≤2 versus >2)	0.7	(0.4–1.2)	0.2

ECOG-PS, Eastern Cooperative Oncology Group performance status; MGMT, O^6-methylguanine-DNA-methyltransferase.

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Table 4
Multivariate analysis (Cox regression model) for overall survival

	Hazard ratio	95% confidence interval	*P*
MGMT status (unmethylated versus methylated)	1.3	(0.8–2.2)	0.3
Time standard TMZ-metronomic TMZ (≤3 versus >3)	0.7	(0.4–1.5)	0.4
ECOG-PS (≤2 versus >2)	0.4	(0.3–0.7)	**0.001**

ECOG-PS, Eastern Cooperative Oncology Group performance status; MGMT, O^6-methylguanine-DNA-methyltransferase; TMZ, temozolomide.

Table 5
Response rates of patients included in the study according to the Response Assessment in Neuro-Oncology (RANO) criteria

	Metronomic TMZ ($n = 120$)
Complete response	0
Partial response	3 (2.5%)
Objective response rate	3 (2.5%)
Stable disease	48 (40%)
Disease control rate	51 (42.5%)
Progressive disease	69 (57.5%)

TMZ, temozolomide.

promoter in nine patients (26.4%), data consistent with other previous studies [32].

In addition, we evaluated patients with different ECOG-PS (range 0–3) and found a strong prognostic impact of ECOG-PS, in contrast to Weller *et al.* [18], where all patients had a good performance status.

The multicentric phase II RESCUE study [19] evaluated the same TMZ schedule analysed in our study (metronomic TMZ administered at 50 mg/m^2/day) in 120 GBM patients, divided into three subgroups in relation to the time of recurrence compared with the end of adjuvant therapy: within six TMZ maintenance cycles; over six cycles but before TMZ maintenance completion; after TMZ maintenance completion and a treatment-free interval of at least 2 months. In this study, a similar median overall survival was observed in patients with and without MGMT methylation (10.3 versus 9.6 months, P = not available), highlighting the absence of a prognostic role for MGMT promoter methylation, similar to our study, although we had a significant association between MGMT methylation status and overall survival at univariate analysis. A possible explanation about MGMT methylation status not being prognostic in our study could be that a relevant part of the patients were evaluated beyond the second line of treatment and that most of them had a previous nitrosoureas exposure before starting metronomic TMZ.

Finally, the population of our study was heterogeneous in number of previous lines of therapy received and ECOG-PS.

Moreover, the RESCUE study's three arms were based on the time of recurrence compared with adjuvant therapy, which has been found to be statistically associated with a better outcome for patients who relapsed within six TMZ maintenance cycles or with a treatment-free interval of at least 2 months, a finding similar to our study, in which patients who had started metronomic treatment within 3 months of maintenance treatment showed increased survival (6.7 months versus 5.3 months, $P = 0.1$).

Regarding the response to treatment, our study achieved an ORR of 2.5% and a high rate of disease control (42.5%); the ORR was lower than that reported in the DIRECTOR (8–16%) and RESCUE (3–11%) studies. These contrasting outcomes may be a result of the heavily pretreated patients enrolled in our study, who received the therapy after two or more lines of prior treatment [17,18].

The metronomic schedule was overall well-tolerated: the main haematological grade 3–4 toxicities were

Table 6
Adverse treatment events based on the CTCAE v.5.0

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3+4	Total ($n = 120$)
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Haematological						
Thrombocytopenia	10 (8.3)	2 (1.6)	3 (2.5)	1 (0.8)	4 (3.3)	16 (13.3)
Neutropenia	5 (4.2)	1 (0.8)	1 (0.8)	1 (0.8)	2 (1.6)	8 (6.6)
Leukopenia	16 (13.3)	1 (0.8)	2 (1.6)	0	2 (1.6)	19 (16)
Lymphocytopenia	13 (10.8)	17 (14.2)	11 (9.2)	1 (0.8)	12 (10)	42 (35)
Anaemia	4 (3.3)	0	0	0	0	4 (3.3)
Non-haematological						
Transaminase increase	10 (8.3)	1 (0.8)	2 (1.6)	0	2 (1.6)	13 (10.8)
Bilirubin increase	2 (1.6)	0	0	0	0	2 (1.6)
Nausea	6 (5)	6 (5)	0	0	0	12 (10)
Vomiting	1 (0.8)	0	0	0	0	1 (0.8)
Fatigue	14 (11.7)	7 (5.8)	1 (0.8)	1 (0.8)	2 (1.6)	23 (19.1)

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Table 7
Clinical studies of alternative temozolomide dosing regimens in patients with newly diagnosed or recurrent gliomas

Reference	Study design	Number of patients	Histology (%)	Temozolomide regimen	Line of treatment	Response		Median overall survival	Any grade 3–4 haematological toxicity (%)
						DCR (%)	ORR (%)		
[16]	Phase II	33	GBM	75 mg/m^2/day (21/28)	2	60	9	4 months	54
[25]	Retrospective	54	HGG	150 mg/m^2/day (7/14)	Second or subsequent	NR	NR	NR	18
[26]	Phase III	422	GBM	75 mg/m^2 (21/28) increased for subsequent cycles to 100 mg/m^2	1	NR	NR	15 months	47
[15]	Phase II	35	28 GBM, 3 AA, 2 AO, 2 oligodendroglioma	75 mg/m^2 days 1–42 q70d	Second or subsequent	6.2	6	8.7 months	NR
[27]	Phase II	37 (28 evaluable)	GBM	50 mg/m^2/day (28/28)	2	36	11	7 months	78
[19]	Phase II	33 Cohort 1	GBM	50 mg/m^2/day (28/28)	2	27.2	3	NR	17
		27 Cohort 2				7.7	0	NR	
		28 Cohort 3				37	11	NR	
[18]	Phase III	Arm A 52	GBM	120 mg/m^2/day (7/7)	2	NR	8	17 months	40
		Arm B 53*		80 mg/m^2/day (21/7)		NR	16	25 months	46
[28]	Phase I	9	Gliomas	50 mg/m^2/day (42/42)	2	NR	NR	NR	11

AA, anaplastic astrocytoma; AO, anaplastic oligoastrocytoma; DCR, disease control rate; GBM, glioblastoma; HGG, high grade glioma; NR, not reported; ORR, overall response rate; OS, overall survival.

* One patient did not receive any study drug.

lymphocytopenia (10%) and thrombocytopenia (3.3%). These data are consistent with the RESCUE study, where grade 3 lymphocytopenia was observed in 15.8% of patients [18]. In the DIRECTOR study, where a different TMZ schedule was used, a higher number of grade 3–4 lymphocytopenia events was found (19.2% and 28.8% of patients in arms A and B, respectively) and a grade 3–4 thrombocytopenia comparable to that observed in our study, with 3.8% of patients in both treatment arms [17].

The only non-haematological grade 3–4 toxicities found in our study were asthenia, with one grade 3 and one grade 4 event (0.8%), and hypertransaminasemia, with two grade 3 events (1.6%). These results are consistent with the DIRECTOR study, where the only non-haematological grade 3–4 toxicity was asthenia (1.9%), but lower than what was recorded in the RESCUE study, where 6.7% and 5.8% of patients had nausea/vomiting and grade 3–4 asthenia, respectively [17,18].

Given that only four of 120 patients (3.3%) in our study discontinued treatment due to toxicity, it can be stated that this regimen was well-tolerated, considering that many patients were heavily pretreated. Due to the retrospective and monocentric nature of the study, we cannot rule out the possibility of a certain degree of toxicity underestimation.

The retrospective nature of our study is an important limitation, as the methodology used for the analysis of the MGMT methylation status has evolved over time, from the initial PCR to pyrosequencing in the last 2 years, with no standardised cut-off for identifying MGMT methylation. In addition, in most patients, methylation status was only determined at the time of diagnosis. Therefore, potential variations in methylation status during disease progression cannot be ruled out.

Conclusions

In our real-life study, rechallenge with TMZ administered continuously with a metronomic schedule at 50 mg/m^2 seemed to be a viable therapeutic alternative for recurrent GBM, even in heavily pretreated patients. Patients with MGMT methylated disease and a good ECOG-PS may benefit more from this treatment.

Ethics

This study was approved by the local institutional review board (Veneto Institute of Oncology Ethics Committee n. 8.2019) and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, with the good clinical practice guidelines and with the Declaration of Helsinki. Written informed consent was obtained from the enrolled patients.

Conflicts of Interest

The authors declare no conflicts of interest.

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Funding

This research received 'Ricerca Corrente' funding from the Italian Ministry of Health (CDC 099183, COMM.SCI: ZAGO-8779/22) to cover publication costs.

Author Contributions

GL is the guarantor of integrity of the entire study. GL was responsible for study concepts and design. AB carried out the literature research. AB and GL carried out the statistical analysis.

AB prepared the manuscript preparation. AB, GC, MC, MP, GL, LD, FC, ADP, VA, VG and VZ edited the manuscript. AB, GC and GL carried out the revision process.

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