

Temozolomide based treatment in glioblastoma: 6 vs. 12 months

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Abstract. The Stupp regimen remains the standard treatment for newly diagnosed glioblastomas, although the prognosis remains poor. Several temozolomide alternative schedules have been studied, with extended adjuvant treatment (>6 cycles of temozolomide) frequently used, although different trials have indicated contrasting results. Survival data of 87 patients who received 6 ('6C' group) or 12 ('12C' group) cycles of temozolomide were collected between 2012 and 2022. A total of 45 patients were included in the 6C group and 42 patients were included in the 12C group. Data on isocitrate dehydrogenase mutation and methylguanine-DNA-methyltransferase (MGMT) promoter methylation status were also collected. The 12C group exhibited statistically significantly improved overall survival [OS; 22.8 vs. 17.5 months; hazard ratio (HR), 0.47; 95% CI, 0.30-0.73; P=0.001] and progression-free survival (15.3 vs. 9 months; HR, 0.39; 95% CI, 0.25-0.62; P=0.001). However, in the subgroup analysis according to MGMT status, OS in the 12C group was significantly superior to OS in the 6C group only in the MGMT unmethylated tumors. The present data suggested that extended adjuvant temozolomide appeared to be more effective than the conventional six cycles.

Introduction

Glioblastoma is the most aggressive and the most frequent brain neoplasia: its incidence is about 5-8 cases per 100,000

inhabitants and represents 54% of all the diagnosed gliomas (1,2). Recent data show a stable incidence in the US or Canada (3), while English and European reports indicate that the incidence is increasing (4).

These tumors are believed to origin from neuroglial or progenitor stem cells and are molecularly heterogeneous (5). The brain tissue microenvironment, including stem cells niches and blood-brain barrier, strongly affects the low rate of glioblastoma metastasis out of the brain, but better promotes brain-invading cancer cells (6).

Molecular profiling has identified three subgroups associated with TERT promoter mutation (7,8): a molecule that plays an important role in cancer formation and in safeguarding chromosomal steadiness by maintaining telomeres' length and has also a role in cellular aging (9). These molecular elements impact tumor growth, avoiding senescence and enabling immortal growth. None of the above-mentioned subtypes are predictive for pharmacological response to present therapies, besides the assignment to subtypes can be difficult because of the intra-tumoral heterogeneity and also the switching subtype is possible through the evolution of disease. Despite the advantages made in our understanding of glioblastoma biology and the current treatment of glioblastoma, including chemotherapy, radiotherapy and surgical approaches, the outcome remains dismal: the median overall survival (mOS) ranging from 14.6 to 20 months (10) and the 5-year survival is less than 10% (11). The treatments fail mainly for the unique molecular features of GBM, particularly due to the presence of a population of stem-like cells called glioma stem cells (GSCs) with ability of self-renewal, making it resistant to current treatments, but also to the presence of blood-brain barrier (BBB) and the privileged immune status (12). For this reason, even a little surgical residue after resection can lead to a lethal recurrence (13). The main weapon following surgery, is the use of the temozolomide (TMZ)-based treatment: this drug is an alkylating agent that better works in methylguanine-DNA-methyltransferase (MGMT)-methylated glioblastomas (14,15). Because of the absence of approved healing treatments, the National

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Comprehensive Cancer Network (NCCN) recommends clinical trials for eligible patients (16) in order to administer tailored treatments basing on age, functional status, goals of care, etc. and to present palliative care earlier in the course of disease (3). If the patient cannot be entered into any clinical trial, the Stupp protocol is the approved standard treatment but roughly 70% of patients will progress within a year and only approximately 27% will be alive at two years (17,18). In Stupp protocol, TMZ can be administered in its conventional schedule (6 cycles) or in its extended schedule (more than 6 cycles). Extended duration of TMZ has been found to be well tolerated, with a low number of major toxicities. Many studies have demonstrated a survival benefit in the extended schedule (mOS 24-31 months) compared to the conventional schedule (mOS 8-16.5 months) (19-22). However, the Spanish Group of Research in Neuro-Oncology (GEINO group) investigated in a phase 2 prospective trial (GEINO 14-01) the optimal duration of TMZ treatment, finding out that extending TMZ after the sixth cycle gave more toxicities and no benefit in 6 months progression free survival (PFS) (23).

Because of these contrasting data, we decided to conduct a bi-centric retrospective analysis to highlight the efficacy of extending adjuvant treatment with temozolomide in patients with glioblastoma.

Patients and methods

Study design and participants. Our study analyzed the effectiveness of extended temozolomide as adjuvant therapy after a first phase of concomitant chemo-radiation in 87 patients diagnosed with glioblastoma. All data were collected retrospectively from two institutions, Azienda Ospedaliera Universitaria Luigi Vanvitelli (Napoli, Italy) and Ospedale Civile 'San Giovanni di Dio' (Frattamaggiore, Italy). Inclusion criteria were those of clinical practice: patients should be 18 years or older, histologically confirmed glioblastoma diagnosis, adequate bone marrow, liver and renal function, stable dose of glucocorticoids with a performance status according to the Eastern Cooperative Oncology Group (ECOG) between 0 and 2. Exclusion criteria were recurrent disease, other metachronous malignancies, need for antiviral treatment for active hepatitis B and C, contemporary use of strong cytochrome P3A4 inhibitors or inducers, treatment discontinuation due to toxicity. We collected data on Isocitrate Dehydrogenase (IDH) mutational status, although the newer WHO classification of CNS (24) tumors define glioblastomas as strictly IDH wild type. We decided to include also these patients based on the initial histological report made at the time of first diagnosis. MGMT methylation was also collected. Both were analyzed on archived tumor tissue, stored in separate laboratories for each center. MGMT methylation status was assessed by methylation array by EPIC array Illumina 850k (25) or Methylation Specific PCR (MSP/PCR) (26), while IDH mutation status was assessed by methylation array by EPIC array Illumina 850k (25) or immunohistochemistry (27). Molecular analysis was not available for all patients as some patients underwent surgery in different centers and, due to the retrospective nature of our study, information were difficult to retrieve.

Procedures. All patients underwent surgical resection or biopsy followed by radiotherapy with concomitant

temozolomide (75 mg/m²/day). After concurrent chemoradiation, treatment was temporarily suspended for the duration of one month and then reprised with adjuvant temozolomide as monotherapy, five days every 28 days: first cycle was administered as 150 mg/m²/day, following cycles as 200 mg/m²/day. The choice to administer six or more cycles was taken by the neurooncologist responsible for the patient based on her/his experience. Brain MRI evaluation was conducted firstly after 40-60 days the last day of chemoradiation and then every three months since the start of temozolomide monotherapy; in case of clinical signs suggestive of progressive disease, brain MRI could be anticipated based on clinician's decision. Tumor progression was defined according to Response Assessment in Neuro-Oncology (RANO criteria). Data were collected until 17th April 2023.

Outcomes. Primary endpoint was OS, defined as time from treatment start to death from any cause, whereas secondary endpoint was PFS, defined as time from treatment start to disease progression or death. PFS2, time from second line start to disease progression or death, was also analyzed. OS, PFS and PFS2 were estimated with Kaplan-Meier methods. Survival data were also stratified according to MGMT methylation status and then excluding IDH mutant tumors. We evaluated the outcomes between 45 patients who discontinued temozolomide therapy at 6 cycles in accordance with the protocol outlined by Stupp *et al* (17) (6C group) and 42 patients wherein TMZ therapy was continued until 12 cycles (12C group). Accordingly, patients who stopped temozolomide before 6 cycles of therapy because of tumor progression or death were excluded from analysis.

Statistical analysis. Patient data were accounted as median with range of minimum and maximum values between parentheses for continuous variables and only percentages for categorical variables. Kaplan Meier estimates were used to help computing survival curves, while survival differences were analyses using the log-rank test, significance level of P=0.05. Statistical analyses were performed using IBM SPSS statistics v.23.0.

Results

Patient's characteristics are summarized in Table I. We included 87 patients with glioblastoma, who received 6 or 12 cycles of temozolomide therapy between 2012 and 2022. Around sixty-five percent (n=56) were male. Median age was 61.6 years (range 31-75). The majority of the patients (83.9%) presented with Eastern Cooperative Oncology Group (ECOG) PS 0-1. Forty-five patients were in the 6C group and forty-two patients in the 12C group. In these 87 patients, MGMT promoter status was known in 56 patients. MGMT promoter was methylated in 44.4% (20/45) and 23.8% (10/42) in the 6C and 12C group respectively. In the remaining 26 patients, MGMT promoter was unmethylated. There was no association between MGMT promoter methylation status and the number of cycles given. As anticipated, we included both IDH wild type and IDH mutant tumors based on initial report made at time of first diagnosis. As expected, the majority (70.1%) were IDH wild type tumors. In only 6 patients IDH was mutated and in 23% the mutational status was instead unknown.

Table I. Population characteristics.

Variable	All patients	6C	12C
Number, n (%)	87 (100.00)	45 (51.7)	42 (48.3)
Median age at diagnosis, years (range)	62 (31-75)	63 (31-73)	60 (32-75)
Male, n (%)	56 (64.4)	25 (55.6)	31 (73.8)
PS 0-1, n (%)	73 (83.9)	36 (80.0)	37 (88.1)
Surgery, n (%)	73 (83.9)	41 (91.1)	32 (76.2)
MGMT-methylated, n (%)	30 (34.5)	20 (44.4)	10 (23.8)
MGMT-unmethylated, n (%) ^a	26 (29.9)	14 (31.1)	12 (28.8)
IDH wild-type, n (%)	61 (70.1)	33 (73.3)	28 (66.7)
Other genomic alterations, n	1	0	1 (BRAF V600E)
Second-line therapy, n (%)	72 (82.8)	42 (93.3)	30 (71.4)

Percentages in the column for all patients are calculated based on the total population. Percentages in the columns for the 6C and 12C groups are calculated based on the total population of the two subgroups. ^aThe methylation status of the remaining patients is unknown. 6C, group of patients that underwent treatment with 6 cycles of adjuvant temozolomide; 12C, group of patients that underwent treatment with 12 cycles of adjuvant temozolomide; IDH, isocitrate dehydrogenase; MGMT, O6-methylguanine-DNA-methyltransferase; PS, performance status.

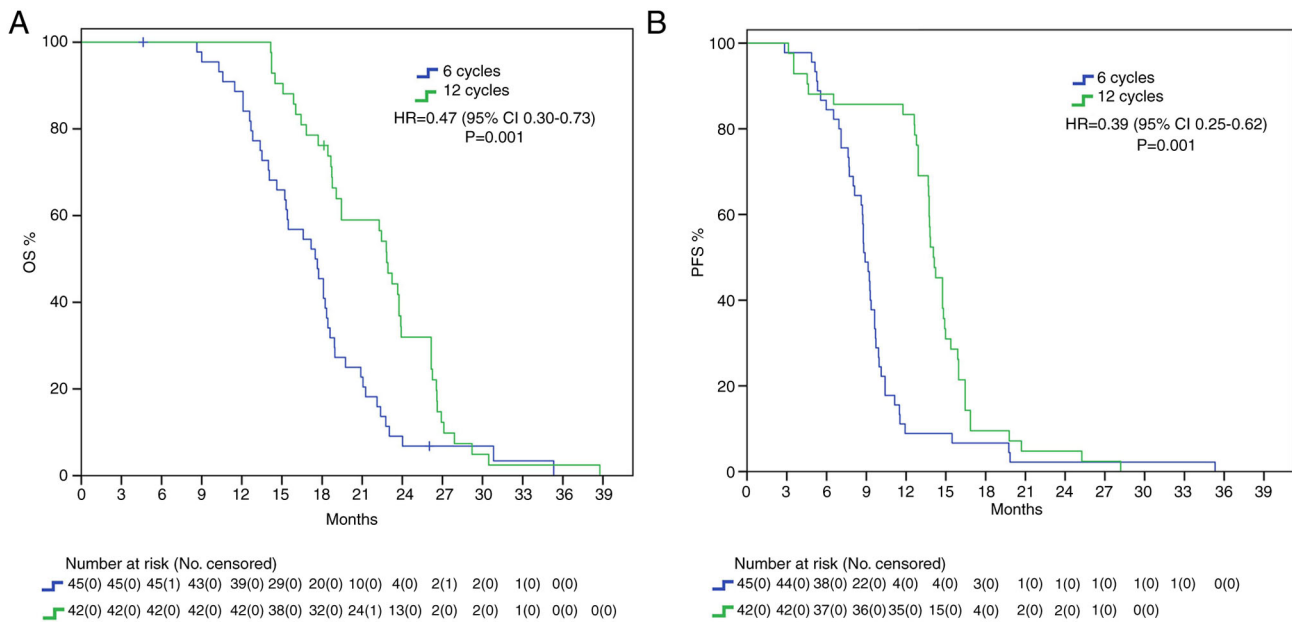


Figure 1. Median (A) OS and (B) PFS in the 6 and 12 cycles groups. HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

Patients whose adjuvant temozolomide therapy was stopped at 6 cycles had a mOS of 17.5 months, whereas those that received 12 cycles reached a mOS of 22.8 months, presenting with a statistically significant benefit (HR 0.47, 95% IC 0.30-0.73 P=0.001). Furthermore, mPFS difference was also statistically significant, with a delta of around 6 months between 12C group and 6C group (15.3 vs. 9 months, HR: 0.39, 95%IC 0.25-0.62, P=0.001) (Fig. 1).

Endpoints were also evaluated in the different subgroups. In MGMT methylated patients, there was a mOS benefit trend in the 12C group (22.8 vs. 18.3 months, HR: 0.52, 95%IC 0.23-1.2, P=0.12) and there was also a positive trend for mPFS (14.8 vs. 8.8 months, HR: 0.49, 95%IC 0.22-1.1, P=0.087) without statistical significance (Fig. 2). Different findings resulted from the analysis in the MGMT unmethylated subgroup: we found a

statistically significant benefit in mOS for the 12C group (22.4 vs. 15.4 months, HR: 0.18, 95%IC 0.063-0.52, P=0.002) but no statistical benefit in mPFS (12.9 vs. 8.8 months, HR: 0.42, 95%IC 0.17-1.02, P=0.056) (Fig. 3).

We repeated our analysis excluding IDH mutant patients, since according to the newest WHO CNS classification they cannot be diagnosed as glioblastomas (24). Nevertheless, here we found a positive trend for mOS (22.4 vs. 17.6 months, HR: 0.62, 95%IC 0.36-1.07, P=0.088) and a significant difference for mPFS (13.7 vs. 9.1 months, HR: 0.42, 95%IC 0.28-0.80, P=0.005) in favor of 12C group (Fig. 4).

Both treatments were generally well tolerated, with a toxicity profile consistent with literature data. Leukopenia was the most frequently observed treatment-related hematologic adverse event, while the most frequent non-hematologic

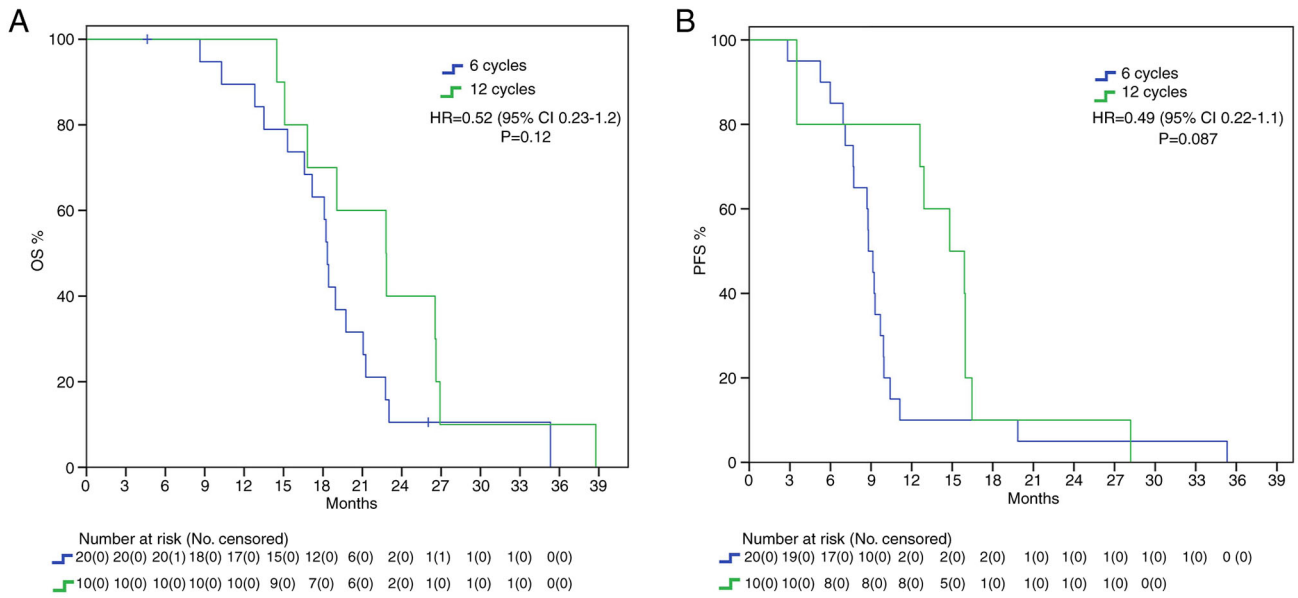


Figure 2. Median (A) OS and (B) PFS in O6-methylguanine-DNA-methyltransferase-methylated patients in the 6 and 12 cycles groups. HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

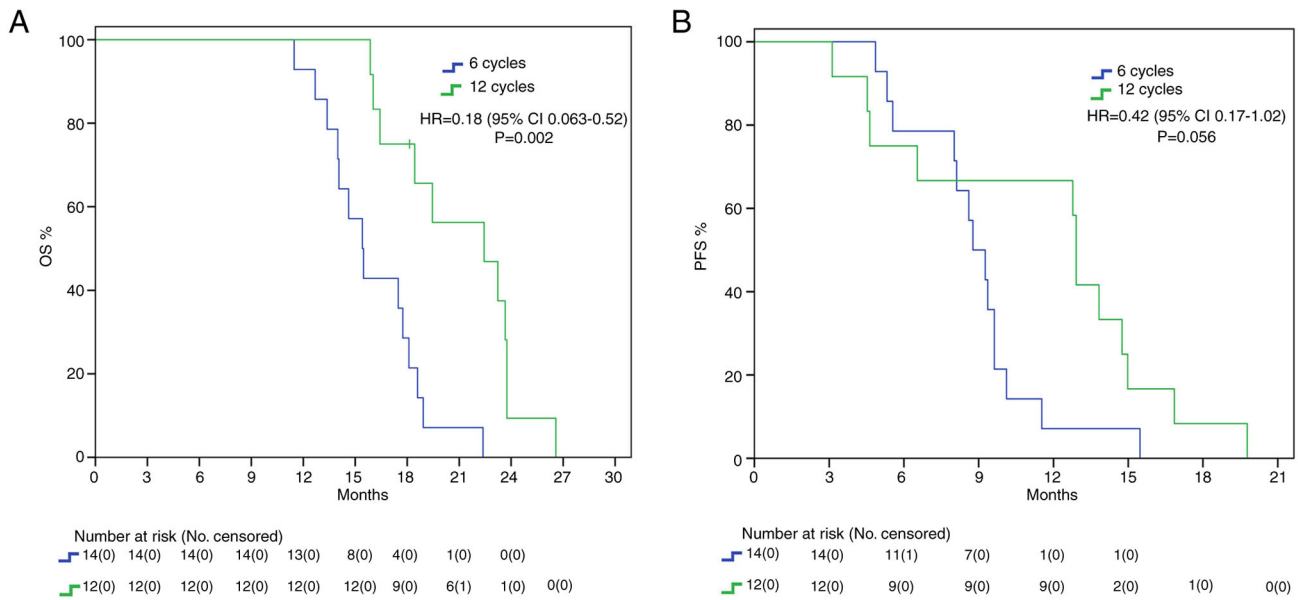


Figure 3. Median (A) OS and (B) PFS in O6-methylguanine-DNA-methyltransferase-unmethylated patients in the 6 and 12 cycles groups. HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

adverse event was fatigue. Overall, in the group of patients treated with the extended schedule of TMZ, there was an increase of adverse events, however it was not necessary to report a statistically significant difference (Table II).

Discussion

The prognosis of patients with glioblastoma remains poor both because, despite after gross total resection there is a high chance of residual disease, and because of the poor efficacy of chemotherapy and radiotherapy treatments. Furthermore, the EORTC 26981/2981/NCIC CE.3 trial (17) allowed only 6 adjuvant cycles of temozolomide, but that was established

as an arbitrary limit. For this reason, adjuvant temozolomide has been prolonged by many investigators, both in everyday practice and in clinical trials, generally up until 12 cycles. Easiness of oral administration and low toxicity profile have also favored prolonging treatment.

While many studies have investigated the benefits of extended adjuvant TMZ (Table III), no definitive indication has been implemented. NCCN guidelines (2023) advice against prolonging treatment (16); European Society for Medical Oncology (ESMO) guidelines, last updated in 2014, do not analyze the controversy; on the contrary, Associazione Italiana di Oncologia Medica (AIOM 2021) guidelines on brain tumor consider the possibility of continuing adjuvant treatment until

Table II. Comparison of the toxicities between patients treated with 6 or 12 cycles.

Adverse event	6 cycles, n (%) (n=45)	12 cycles, n (%) (n=42)
Leukopenia	10 (22)	11 (26)
Neutropenia	9 (20)	9 (21)
Anemia	3 (7)	3 (7)
Thrombocytopenia	4 (9)	5 (12)
Fatigue	14 (31)	18 (43)
Nausea	13 (29)	13 (31)
Constipation	4 (9)	4 (10)
Pneumonia	2 (4)	2 (5)
Hepatotoxicity	4 (9)	3 (7)
Headache	5 (11)	7 (17)
Dizziness	2 (4)	5 (12)

12 cycles (28). We found a statistically significant benefit in the 12C group both in mOS (22.8 vs. 17.5 months, HR 0.47) and in mPFS (15.3 vs. 9 months, HR 0.39) in the overall population, with around 5 months delta in both setting. It must be noted that our survival data are in range with expectations from known literature and thus do not classify as outlier. While mPFS curves seem to cross early on, casting benefit of extended adjuvant treatment in regard to mPFS, the curves tend to grow apart with time, showing its survival benefit. We further analyzed our results considering MGMT methylation status to consider the possibility of only MGMT methylated GBMs having increased survival from extended therapy. Our results show that this subgroup of patients presents with a trend in increased mOS and mPFS in the 12C group. This is in line with the well-known role of MGMT promoter methylation as a predictive factor of increased response to alkylating agents (29,30). It must be said that MGMT methylation determines better prognosis, and it has been speculated that increased survival allows patients to receive more extended treatment (31). Furthermore, mutational changes due to prolonged temozolomide, especially in patients with absence of MGMT-mediated DNA repair, may promote tumor resistance thanks to the acquisition of an alkylating agents-resistant phenotype (32,33). The phase II RESCUE trial on continuous dose-intense temozolomide in recurrent GBM demonstrated worse results in those patients that experienced progression while on extended treatment, while increased survival was found in those with at least a 2-month treatment free interval or experiencing progression on standard treatment (34). In contrast with these results, no mPFS benefit was found in the unmethylated cohort, with only a small trend in increased survival (12.9 vs. 8.8 months). Instead, mOS was found to be statistically significantly higher in the 12C group than in the 6C group (22.4 vs. 15.4 months). We speculated that the increase in OS may be due to second line treatment. About 99.7% of patients in 12C group and 87.5% in 6C group underwent second line therapy. However, no difference was found in PFS2 between the two group. IDH mutant gliomas are characterized by increased survival and tumor response (35).

However, whereas the 2016 classification allowed for IDH wild type and IDH mutant GBMs, the newest 2021 WHO classification of CNS tumors classify GBM strictly as IDH wild type, IDH mutant being astrocytoma or oligodendroglioma according to 1p-19q codeletion status (24). We included in our analysis 6 patients who presented with mutation in IDH1 or IDH2 and 20 with unknown alterations. Nevertheless, we decided to include these patients due to the fact that, at the time of diagnosis, they were classified as GBM. We then repeated mOS and mPFS analysis excluding these 26 patients, limiting our scope accordingly to the newest definition of GBM. Even stratifying according to the newest definition, we confirmed a statistically significant difference in mOS (22.4 vs. 17.6 months) and mPFS (13.7 vs. 9.1 months) in the 12C arm compared to the 6C arm. IDH-based classification is a fundamental game-changer in CNS research. Many previous trials on GBMs often included IDH mutant GBMs, misclassified and now considered a different entity, thus limiting their interpretations. For example, Chen *et al* (36) study on extended temozolomide reported in a retrospective cohort an increased difference in mOS between the extended adjuvant cohort and the control group, around 9.3 months (29 vs. 16.7 months). However, looking at population characteristics, only 27.5% patients in the control group were IDH mutant against 43.4% in the extended adjuvant group, increasing the chance of a higher survival in the latter arm. Indeed, survival benefit was even higher in the IDH1 mutant subgroup (+20.5 months), while there was only a 7-months difference between the two arms in IDH1 wild type subgroup.

Our study is in line with previous analyses demonstrating increasing benefit from extended therapy (Table III) (19,22,36,37). Our results suggest, then, that extended adjuvant treatment may be a good therapeutic opportunity in fit patients to increase survival rates. Rigorous patient selection is of course needed, and while MGMT methylation may take the spotlight, several other factors may influence treatment choice. Keeping in mind the limitations of Chen study, they showed an increased mPFS in newly diagnosed GBMs with higher expression of Ki67 treated with extended adjuvant temozolomide, while no such difference was found in patients with lower Ki67 expression. No difference was found in mOS as both groups benefitted from extended adjuvant treatment (36). A retrospective analysis by Bocangel *et al* (38) evaluated the role of p53 status, since literature reported that p53 mutational and expression status was associated to GBM prognosis. Indeed, wild type p53 was found to inhibit MGMT expression, potentially increasing the response rate to alkylating agents. In Malkoun *et al* (20), p53 overexpression was associated with improved mPFS, even though contrasting results are available in literature (39-41). Furthermore, the study by Skardelly *et al* (42) published in 2017, while only demonstrating a benefit for prolonged temozolomide only for mPFS and not in mOS, found that MGMT status, extent of resection and age are significant covariates for survival analysis.

Reports on toxicity with extended treatment are contrasting. It is necessary to clearly whether prolonged therapy impacts on toxicity and consequently on the quality of life of patients. Quality of life still represents a primary objective to be pursued

Table III. Studies on extended adjuvant TMZ.

First author/s, year	Treatment	Survival results	(Refs.)
Chen <i>et al</i> , 2022	TMZ + RT→TMZx6 or x >6	ITT and methylated improved OS and PFS; unmethylated improved OS, longer PFS; IDH1 mutant presented with bigger delta: More suitable to extended TMZ	(36)
Balana <i>et al</i> , 2020	TMZ + RT→TMZx6 or x12	No benefit in OS and PFS in general population, in MGMT methylated and in pts with measurable disease	(23)
Hau <i>et al</i> , 2007	TMZ + RT→TMZx >6	Increased 2-year OS in primary GBM group with prolonged TMZ	(21)
Bhandari <i>et al</i> , 2017	TMZ + RT→TMZ x6 or x12	Prolonged OS and PFS, but no statistical significance	(54)
Skardelly <i>et al</i> , 2017	TMZ + RT→TMZ x6 or x >6	Prolonged OS and PFS, but no statistical significance. MGMT status, EOR, age were significant covariates for survival	(42)
Blumenthal <i>et al</i> , 2017 ^a	TMZ + RT→TMZ ^b	Prolonged PFS, but no statistical significance. Benefit enhanced in MGMT-methylated group but lost in MGMT-unmethylated; no OS benefit	(33)
Refae <i>et al</i> , 2015	TMZ + RT→TMZ x6 or x >6	Better OS and PFS with extended TMZ	(22)
Darlix <i>et al</i> , 2013	TMZ + RT→TMZ x6 or x >6	Increased OS and PFS with extended TMZ	(37)
Roldán Urgoiti <i>et al</i> , 2012	TMZ + RT→TMZ x6 or x >6	Increased OS and PFS in extended TMZ	(19)

^aPooled analysis. ^bBoth TMZ/RT->TMZ and experimental arms were used, since none of the experimental arms demonstrated significant benefit. GBM, glioblastoma; IDH, isocitrate dehydrogenase; ITT, intention-to-treat population; MGMT, O6-methylguanine-DNA-methyltransferase; mOS, median overall survival; OS, overall survival; PFS, progression-free survival; TMZ, temozolomide; EOR, extent of resection; RT, radiotherapy.

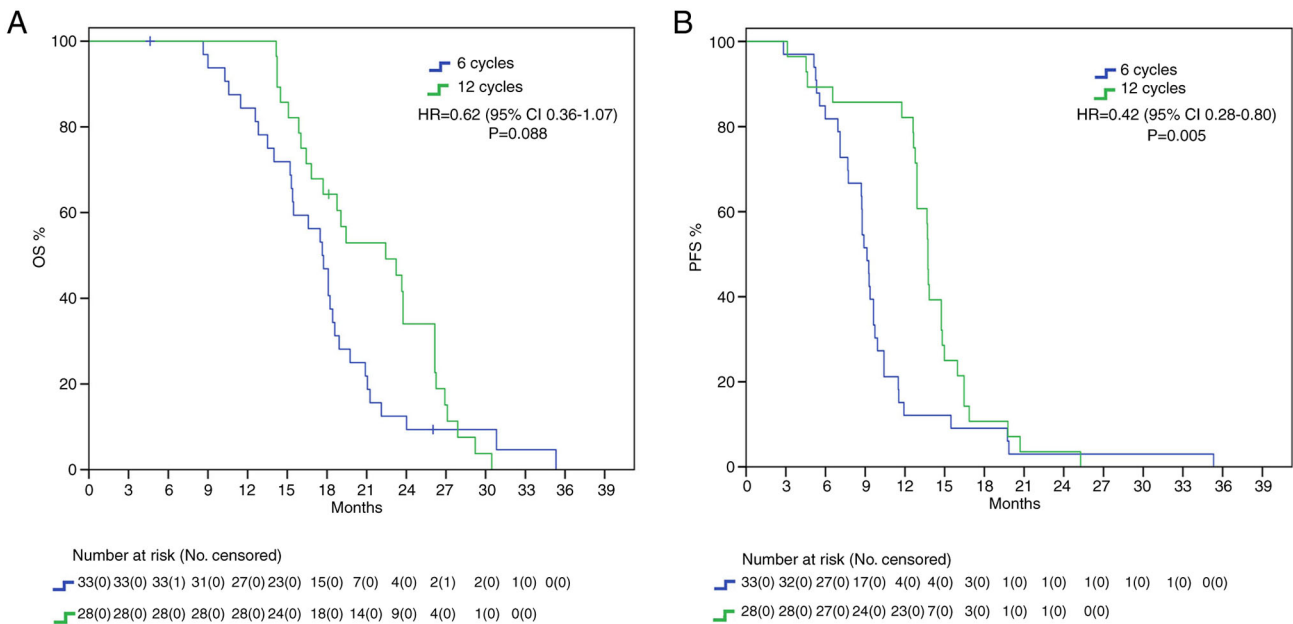


Figure 4. Median (A) OS and (B) PFS in isocitrate dehydrogenase wild-type patients in the 6 and 12 cycles groups. HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

today since we cannot yet aim for cure. Only clinical data derived from a randomized study can disprove the common sensation that extended treatment is accompanied by greater

toxicities, particularly hematological. In clinical practice only a limited percentage of patients manage to have prolonged treatment (43). The safety analysis of the Prolonged Adjuvant

Temozolomide vs. ‘Stop & Go’ in Glioblastoma Patients (PATSGO) trial on 34 patients demonstrated that frequency of toxicity did not increase with number of cycles (44); instead, in the GEINO trial, lymphopenia, thrombocytopenia, nausea and vomiting were more frequent in the extended therapy group, although few patients experienced grade 3-4 adverse events of any kind and only three patients (3.7%) needed to discontinue treatment (23). However, there are other several reports of increased toxicity with prolonged temozolomide administration: increased cumulative doses of temozolomide have been associated with worse quality of life and fatigue (10,45), risk of myelosuppression and immunodepression (46), myelodysplasia and even leukemia (47).

As already mentioned before, while no consensus exists on the benefits of additional temozolomide, most physicians settle at a maximum of 12 cycles for maintenance therapy, trying to find balance between possible beneficial effects and toxicities. A single center study by Ohno *et al* (48) compared stopping treatment at 12 cycles or proceeding beyond 12 cycles. mPFS and mOS between the two groups demonstrated no difference (mPFS 11.3 vs. 9.2 months, mOS 25.7 vs. 30.2 months), with only Karnofsky performance status at 12 cycles having a significant association with increased survival (48).

Temozolamide treatment has also been associated with induced hypermutation. No data exists on the perfect treatment schedule or duration in order to reach the most benefit while reducing the risk of induced hypermutation and toxicity (49). While conferring resistance to temozolomide treatment (34), these changes may help identify new treatment strategies for recurrent/progressing GBM. Hypermutation seems to present with an increased sensitivity to DNA-damaging agents (50), with preclinical trial demonstrating improved sensitivity to lomustine in mismatch repair (MMR) deficient MGMT methylated GBM cells resistant to temozolamide (51). It has also been speculated that hypermutated cancer cells may be more responsive to immune checkpoint inhibition (52) but results from nivolumab trials both in newly diagnosed GBM and in recurrent GBM have demonstrated poor results. Pembrolizumab is now under investigation in patients with recurrent gliomas with hypermutator phenotype (NCT02658279) but a recent monocentric study by Lombardi *et al* (53) found no apparent benefit.

Of course, our study presents several limitations. It is a retrospective analysis, with only a modest sample size (87 patients), thus limiting extrapolation of its results. The study was of course not randomized and no information regarding treatment choice (6 vs. 12 cycles) is available, with the possibility of selection bias. MRIs at progression were not centrally reviewed, in line with the nature of the study, and recorded toxicity data was limited, not allowing for further study. OS data may also be influenced by second-line choices (mainly fotemustine and regorafenib). However, the number of patients enrolled and the results obtained in our study are substantially similar with that has already been published by Bhandari and colleagues (54).

In conclusion, our data suggests that extended adjuvant temozolomide (12 cycles) appears to be significantly more effective than standard treatment with only conventional six cycles. While literature data are quite heterogeneous and do not provide any strong evidence for stopping or continuing

temozolomide, in the absence of larger phase III trials, continuing adjuvant temozolomide for more than six cycles may be an effective alternative.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MF, FC and RA conceived the study. MF and RA described the methodology to be used. Investigation was done by MP, CCM, SF, AZ, VF, PV, IDG, CB, VC, DS, TS, LMC, PC, MC and RP. VDF, VF, PV, IDG, CB, VC, DS, TS, LMC, PC, MC and RP analyzed the data. Validation of data was performed by VDF, VF, PV, IDG, CB, VC, DS, TS, LMC, PC, MC and RP. The resources were collected by MF, VF, PV, IDG, CB, VC, DS, TS, LMC, PC, MC, RP, FC and RA. Data was curated by VDF, VF, MP and SF. The original draft was prepared by VDF, MP, CCM, SF and AZ. The final text was reviewed by MF, VDF and RA. Work was supervised by MF and RA. MF and RA confirm the authenticity of all the raw data. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

All subjects gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki. The retrospective study protocol was approved by the institutional review board of University of Campania Luigi Vanvitelli (Napoli, Italy; protocol no. 59;).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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