Radiotherapy and sequential temozolomide compared with radiotherapy with concomitant and sequential temozolomide in the treatment of newly diagnosed glioblastoma multiforme

Vitaliana De Sanctis[a], Giorgio Mazzarella[a], Mattia F. Osti[a], Maurizio Valeriani[d], Marco Alfo'[b], Maurizio Salvati[c], Enzo Banelli[a], Vincenzo Tombolini[d] and Riccardo Maurizi Enrici[a]

Our objective was to determine and compare effects of sequential temozolomide vs. concomitant plus sequential temozolomide with conventional radiotherapy, in patients with newly diagnosed glioblastoma multiforme, comparing two independent trials. Sixty-four patients were treated on two consecutive separate phase II studies that used identical eligibility criteria and the same radiotherapy (60 Gy, 2 Gy/day, after surgery) and adjuvant temozolomide (200 mg/m^2/day for 5 days/28 days until progression), but differed in the absence or presence of a concomitant treatment with temozolomide (75 mg/m^2/day) during radiotherapy. In the first protocol (1999–2002), 21 patients (median age of 64 years) received radiotherapy alone and sequential temozolomide; in the succeeding protocol (2002–2004), 43 patients (median age of 61 years) with similar characteristics received radiotherapy with concomitant and sequential temozolomide. Median number of adjuvant cycles was five in both trials. Median survival was similar in both studies (18 vs. 17.4 months); overall survival rates at 6, 12, 18 and 24 months of all the population were 89, 69, 45 and 24%. No statistically significant differences were found among prognostic factors considered. Hematologic toxicities were mild and similar, with grade 3–4 neutropenia in 5–7% and grade 3–4 thrombocytopenia in 7–10% of patients in the sequential phases, and grade 3–4 thrombocytopenia in 7% in the concomitant phase of temozolomide. We confirmed that temozolomide combined with radiotherapy is well tolerated and provides a survival advantage compared with historical data using radiotherapy alone. Nevertheless, a concomitant use of temozolomide during radiotherapy does not seem to improve survival, although it does not increase toxicity. *Anti-Cancer Drugs* 17:969–975 © 2006 Lippincott Williams & Wilkins.

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Departments of [a]Radiation Oncology, [b]Statistics and [c]Neurosurgery, University 'La Sapienza', Rome and [d]Department of Radiation Oncology, University of L'Aquila, L'Aquila, Italy.

Correspondence to V. de Sanctis, Department of Radiation Oncology, University 'La Sapienza', via di Grottarossa 1035/1039, 00189 Rome, Italy.
Tel: +39 06 80345601; fax: +39 06 80345610;
e-mail: vitaliana.desanctis@uniroma1.it

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Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive type of glioma, representing about 50% of all gliomas (which account for 1–2% of all adult tumors). Current first-line treatment protocols infrequently achieve long-term survival. Maximal surgical resection is currently the standard of care; the role of radiotherapy (RT) in prolonging overall survival (OS) has been proved in several clinical trials [1], increasing median survival from 4–5 months [2] to approximately 9–12 months [3], with a 2-year survival rate of 9% [4]. The role of adjuvant chemotherapy has been largely investigated: although the meta-analysis of Stewart [4] reports an increase in the 2-year survival rate to 13%, several randomized clinical trials have shown divergent results with regard to a substantial benefit of various chemotherapeutic agents, particularly nitrosoureas [5–7], despite a considerable myelosuppression.

In the last decade, the role of the DNA-methylating agent temozolomide (TMZ) emerged, with the aim of chemotherapy-related improvement of median survival and of quality of life. TMZ was proved to have good penetration in the cerebrospinal fluid [8] and to be effective in recurrent malignant gliomas and better tolerated than nitrosoureas in several clinical trials [9–11]. The dose of 200 mg/m^2/day for 5 consecutive days every 28 days is the approved schedule, although the drug is supposed to have a particular schedule-dependent cytotoxicity with theoretical advantages for protracted administrations [12,13]. An extended schedule enables a greater dose of TMZ to be administered per unit of time, with a supposed inhibition of O^6-alkylguanine DNA-alkyl transferase (AGAT), one of the major mechanisms of resistance [14]. Moreover, some preclinical data have suggested possible interactions between TMZ and radiation. In-vitro studies, by Wedge *et al.* [15] with the

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human U373MG GBM cell line and by Trog *et al.* [16] with the human U87MG GBM cell line, showed an increased cytotoxicity, whereas the study by van Rijn *et al.* [17] displayed an increased cytotoxicity in the human D384 astrocytoma cell line, but not in the human U251 GBM cell line.

The mechanism of action of TMZ and its activity against recurrent high-grade gliomas were the rationale of combining the drug with RT and using it as an adjuvant agent, as first-line therapy for GBM. Furthermore, the use of a protracted schedule with a continuous daily administration of TMZ, as an alternative to the approved schedule, prompted us to use 75 mg/m^2 daily for 6–7 weeks during the period of RT. A phase II study conducted by Stupp *et al.* [18] showed the safety and the efficiency of concomitant RT and TMZ (75 mg/m^2/day) followed by adjuvant TMZ (200 mg/m^2/day for 5 days every 28 days, for a maximum of six cycles), reporting an increase to 15.8 months of the median survival, calculated from the time of beginning of RT. The European Organization for Research and Treatment of Cancer and the National Cancer Institute of Canada have conducted a phase III randomized trial in which they compared this regimen with RT alone. Conclusive results confirm a longer median survival of 14.6 months for the group treated with concomitant RT plus TMZ followed by adjuvant TMZ, compared with a median survival of 12.1 months for the group treated with RT alone [19]. This is the largest randomized study to demonstrate a statistically significant improvement in survival using a chemotherapeutic agent in GBM, establishing a new standard of care. Another recently published smaller randomized study by Athanassiou *et al.* [20] demonstrates that RT with concurrent TMZ followed by adjuvant TMZ is more effective than RT alone (median survival of 13.4 vs. 7.7 months) in patients with newly diagnosed GBM; the concomitant phase of the treatment was equal, but the adjuvant phase was slightly different (150 mg/m^2 of TMZ on days 1–5 and 15–19 every 28 days, for a maximum of six cycles). Although both these papers demonstrate the benefit of chemotherapy in prolonging survival, it is not investigated whether this improvement is a result of the adjuvant phase of treatment with a maximum of six cycles of TMZ or of the continuous daily administration of TMZ during RT. In addition, when we consider the concomitant RT and TMZ phase, it should be taken into account whether the result is due to a prolonged schedule of TMZ or to a synergistic effect with RT.

At the two participating institutions (Universities of Rome and L'Aquila), we conducted two consecutive prospective phase II trials in patients with newly diagnosed GBM. Each trial used the same radiotherapeutic protocol and sequential treatment with TMZ (approved schedule of 200 mg/m^2/day for 5 days every 28 days, until progression), but only the second trial included the concomitant treatment with TMZ during RT. Results from the phase II study by Stupp *et al.* [18] led us to close the first trial and open the second trial, with a more intensive chemotherapeutic treatment. Comparison of the results of these two trials may provide information as to whether concurrent chemo-RT with TMZ can contribute to improve survival.

Methods

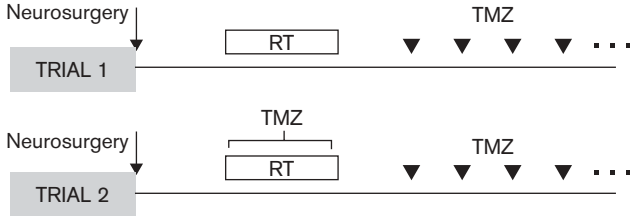
Eligibility

Patients eligible for the studies were required to be at least 18 years old and to have a histologically confirmed diagnosis of GBM, classified according to the World Health Organization. All diagnoses were performed or reviewed by the two neuropathologists of the two participating centers. All patients had previously undergone neurosurgical treatment (biopsy, partial resection or complete resection) and none of them had to have undergone antineoplastic chemotherapy (except corticosteroids) before entering the studies, and in the period between neurosurgical treatment and RT. Patients were required to have a surgical procedure ≤ 6 weeks before the start of RT, Karnofsky performance status (KPS) > 50, life expectancy > 3 months, adequate bone marrow function (peripheral absolute granulocyte count > 1500/mm^3, hemoglobin > 10 g%, platelet count $> 100\,000$/mm^3), and normal baseline liver and renal functions. Patients who were of potentially child-bearing age were advised to practice adequate contraception. The pretreatment evaluation included a complete medical history, and a physical and neurological examination, with a KPS determination. The nature and purpose of the studies were fully discussed with each patient, and each provided written informed consent before registration. The protocols were approved by the Clinical Research Ethics Committees of the Universities of Rome and L'Aquila.

Treatment

External beam RT was administered according to the guidelines of the International Commission on Radiological Units (ICRU 62), with identical modalities in both trials. Patients were positioned and immobilized with individual masks. Target volumes were defined comparing preoperative and postoperative gadolinium-enhanced magnetic resonance imaging (MRI) of the brain. Planning target volume was considered the contrast-enhancing mass on T1-MRI, with margin of 2–3 cm; only for larger tumors or to preserve organs at risk we considered a boost (after 40–50 Gy), excluding edema. After three-dimensional computed tomography-based treatment planning, RT was delivered with linear accelerators (6 MV photon energy or more) using a multileaf collimator. The daily dose was 2 Gy for 5 days each week to a total dose of 60 Gy.

Fig. 1



Schemes of the two consecutive schedules. RT, radiotherapy; TMZ, temozolomide.

Patients enrolled in the first trial (within April 2002) received adjuvant TMZ 4 weeks after RT: 200 mg/m^2/day for 5 consecutive days every 28 days until radiological or clinical progression of the tumor. Patients enrolled in the second trial (since April 2002) received concomitant TMZ during RT: 75 mg/m^2/day for 7 days, 1 h before RT and on days of the week without RT; adjuvant TMZ 4 weeks after RT was administered with the same schedule as the first trial (200 mg/m^2/day for 5 days every 28 days). Patients always received TMZ in a fasting state and did not eat for at least 2 h after receiving TMZ (Fig. 1). Patients received corticosteroids, and mannitol therapy as needed, at the lowest dose necessary to maintain neurological stability before and during RT; supportive therapy was discontinued, if possible, after RT. Anticonvulsants were given when indicated. Antiemetics were prescribed with TMZ as needed.

Response and toxicity evaluation

The end points of this study were evaluation of the OS and the toxicity, related at the two consecutive protocols.

A gadolinium-enhanced MRI was performed before RT, 1 month after completion of RT and then every 2–3 months. All MRIs were reviewed by two neuroradiologists of the two participating centers. GBM were classified as T1 if the maximum diameter was equal to or less than 5 cm, T2 if more than 5 cm, T3 if invasion of the ventricular system was present and T4 if extension beyond the midline or infratentorially was present.

Toxicities were graded according to the National Cancer Institute's Common Toxicity Criteria (version 2.0). Complete blood count and serum chemistry were monitored before RT and weekly during RT, or more frequently if indicated. A complete blood count was monitored before each cycle of adjuvant TMZ and once or twice in the 28 days.

After the progression of the disease, patients were treated according to the single case.

Statistical analysis

OS was calculated from the time of surgical procedure (time of histologic diagnosis) until death or last follow-up. Survival curves were estimated using the Kaplan–Meier method [21] with SPSS statistical software and expressed as median survival; they were reported at 6, 12, 18 and 24 months. We considered as prognostic factors the following: age (≤ 50 vs. > 50 years), KPS at diagnosis (> 70 vs. ≤ 70), dimension and feature of the tumor at diagnosis (T1 vs. T2, T3 and T4), extent of surgical resection (gross total tumor resection vs. partial resection and biopsy) and protocol of TMZ administration (adjuvant vs. concomitant plus adjuvant). The univariate and, if appropriate, multivariate statistical analysis was carried out by the logistic-linear Cox regression model. The comparison of survival curves was performed with the log-rank test for censored data [22,23]; a Wilcoxon (Breslow) test for the equality of survivor functions [24,25] was also used. Finally, an additional statistical analysis was performed comparing patients of this study with those of a historical database of 29 patients with GBM treated with RT alone, after neurosurgery, in the Universities of Rome and L'Aquila. Patients were well matched for the prognostic factors of age at diagnosis, KPS, T stage and extent of surgical resection (retrospective analysis of patients treated between October 1997 and November 1999, without any kind of first-line treatment after neurosurgery and RT).

Results

Characteristics of the patients

Between November 1999 and January 2004, a total of 64 patients were enrolled at the two participating institutions in both trials. On the first therapeutic protocol, conducted from November 1999 to April 2002, 21 patients received only sequential TMZ after RT; on the second therapeutic protocol, conducted from April 2002 to January 2004, 43 patients received both concomitant TMZ with RT and sequential TMZ after RT. Patients' characteristics of the two groups are shown in Table 1. The median age at diagnosis of the entire population was 61.5 years (range 31–80 years), KPS before RT was greater than 70 in 37 patients (58%), tumor size was classified as T1 in 33 cases (52%) and 39 patients (61%) underwent a gross total tumor resection, as estimated by the neurosurgeons (early postoperative computed tomography or MRI was not mandatory). At the pathological examination, the value of the proliferation marker Ki-67 (detected by the originally discovered antibody or by MIB1) ranged from 12 to 55 (median 24) in the first trial and from 5 to 40 (median 15) in the second trial. No major differences were present in the pretreatment characteristics of the two groups; however, with regard to age, slightly more patients were older than 50 years in the first trial than in the second trial (95 vs. 79%). All 64 patients completed the planned RT without protocol violations, and all were assessable for survival and toxicity.

Table 1 Characteristics of patients

	Trial 1	Trial 2	Historical cohort
No. of patients	21	43	29
Sex: male/female	13/8	25/18	14/15
Median age (years)	64	61	65
Range (years)	46–80	31–78	34–78
Age $\leq$ 50 years = [*n* (%)]	1 (5)	9 (21)	4 (14)
KPS > 70 = [*n* (%)]	10 (48)	27 (63)	13 (45)
Tumor size = [*n* (%)]			
T1	9 (43)	24 (56)	13 (45)
T2	6 (29)	10 (23)	8 (27)
T3	2 (9)	3 (7)	2 (7)
T4	4 (19)	6 (14)	6 (21)
Neurosurgery = [*n* (%)]			
Total resection	14 (67)	25 (58)	19 (66)
Partial resection	7 (33)	15 (35)	9 (31)
Biopsy	–	3 (7)	1 (3)

KPS, Karnofsky performance status

Table 2 Hematologic toxicity = [*n* (%)]

	Trial 2		Trial 1
	TMZ concomitant	TMZ adjuvant	TMZ adjuvant
Grade 3/4 thrombocytopenia	3 (7)	3 (7)	2 (10)
Grade 3 thrombocytopenia	2 (5)	2 (5)	1 (5)
Grade 4 thrombocytopenia	1 (2)	1 (2)	1 (5)
Grade 3/4 neutropenia	–	3 (7)	1 (5)
Grade 3/4 lymphocytopenia	13 (30)	–	–

TMZ, temozolomide.

The baseline characteristics of the two trials are compared with the baseline characteristic of the historical cohort in Table 1.

After radiological or clinical progression, 12 patients (57%) did not undergo any second-line treatment after TMZ in the first trial and 19 patients (44%) did not undergo any second-line treatment in the second trial.

Toxicity

In the first trial, the major toxicity of the adjuvant TMZ was hematologic: grade 3–4 neutropenia occurred in one patient (5%), grade 3–4 thrombocytopenia in two patients (10%), with one patient experiencing grade 4. In all patients, experiencing grade 3–4 thrombocytopenia or grade 4 neutropenia, the following cycle was delayed, but no dose reduction was adopted. In the second trial, the major toxicity was, once again, hematologic, during the concomitant RT plus TMZ phase: no grade 3–4 neutropenia occurred, grade 3 thrombocytopenia was experienced by two patients (5%, with interruption of the TMZ therapy but not of RT), grade 4 thrombocytopenia was experienced by one patient (2%, with interruption of both TMZ and RT and platelet transfusion), grade 3–4 lymphocytopenia occurred in 13 patients (30%); in the adjuvant phase of TMZ, the incidence of hematologic toxicity was similar to that reported in the first trial: three patients with grade 3–4 neutropenia (7%), three patients with grade 3–4 thrombocytopenia (7%), with a delay of the succeeding cycle in all patients experiencing grade 3–4 thrombocytopenia or grade 4 neutropenia. Hematologic toxicities encountered in both trials are summarized in Table 2.

Non-hematologic toxicity consisted mainly of nausea (prophylactic antiemetics were prescribed once a day before adjuvant TMZ and prescribed for no longer than 3 weeks in the concomitant phase, excepting four patients who required antiemetics for the entire period); moderate skin rash occurred in two patients during the concomitant phase.

Survival

After RT, patients of the first trial received a median of five cycles of TMZ (range 1–13); in the second trial, patients received a median of five cycles of adjuvant TMZ (range 1–17). For the entire population of both protocols, the median survival of the 64 patients treated with RT and TMZ was 17.4 months [95% confidence interval (CI), range 14.6–20.3 months], with OS rates at 6, 12, 18 and 24 months of 89, 69, 45 and 24%, respectively. According to the prognostic factors considered (age $\leq$ 50 vs. > 50 years, KPS > 70 vs. $\leq$ 70, tumor classified as T1 vs. others, gross total tumor resection vs. partial resection and biopsy), a significant improvement in survival was observed only in the subgroup of patients with a tumor classified as T1 ($P = 0.049$), whereas a trend was encountered in patients with gross total tumor resection and KPS > 70.

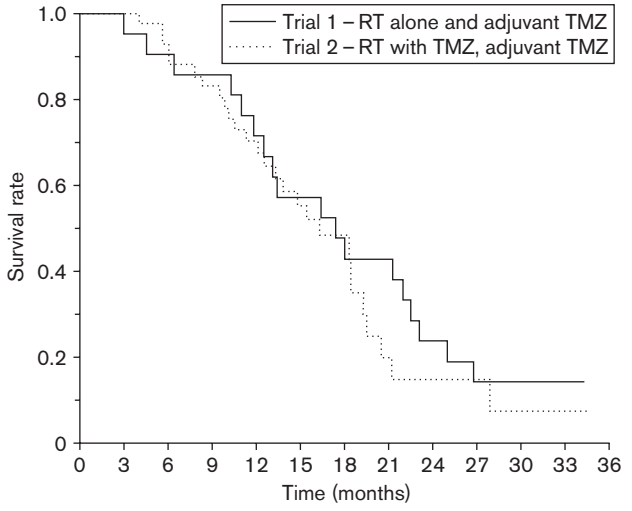
In the first trial (patients treated with TMZ only in the adjuvant phase after RT), the median survival was 18 months, on the basis of Kaplan–Meier estimates (95% CI, 10.8–25.3 months); the OS at 6, 12, 18 and 24 months was 90, 71, 47 and 28%, respectively; the median progression-free survival was 8.9 months. In the second trial (patients treated with TMZ either during RT or in the adjuvant phase after RT), the median survival was 17.4 months (95% CI, 12.9–21.9 months); the OS at 6, 12, 18 and 24 months was 88, 67, 44 and 20%, respectively; the median progression-free survival was 8.5 months. The slight prevalence (although not statistically significant) of older patients (> 50 years) in the first trial was not considered a bias, because it was present in the group of patients with the less aggressive treatment and expected to have the worse survival. Table 3 summarizes survival data in the context of treatment protocol, age, KPS, T stage and extent of surgery. No

Table 3 Survival in the context of the stratification factors

	No. of patients	Median survival (months)	6-month OS (%)	12-month OS (%)	18-month OS (%)	24-month OS (%)	*P*
All patients	64	17.4	89	69	45	24	
Trial 1	21	18	90	71	47	28	0.43
Trial 2	43	17.4	88	67	44	20	
Age >50 years							
Trial 1	20	18	90	70	50	30	0.56
Trial 2	34	15.7	88	69	39	26	
KPS >70							
Trial 1	10	18	90	70	50	30	0.68
Trial 2	27	19.5	88	71	53	26	
KPS ≤ 70							
Trial 1	11	16.4	90	63	45	27	0.38
Trial 2	16	13.9	87	61	30	10	
T1							
Trial 1	9	22	100	88	55	44	0.18
Trial 2	24	17.4	82	73	41	27	
T2, T3, T4							
Trial 1	12	13	81	54	45	18	0.38
Trial 2	19	13.6	93	57	49	3	
Total resection							
Trial 1	14	22	92	78	57	42	0.21
Trial 2	25	17.4	87	70	42	26	
Partial resection and biopsy							
Trial 1	7	13.1	85	57	14	3	0.68
Trial 2	18	15.7	88	63	46	11	

OS, overall survival; KPS, Karnofsky performance status.

Fig. 2



Kaplan–Meier survival curves for patients of the two trials. RT, radiotherapy; TMZ, temozolomide.

comparison was performed in the subgroup of patients less than 50 years old because only one patient ≤ 50 years of age was present in trial 1. From the comparison of the two trials, survival was similar between patients treated with RT alone and adjuvant TMZ and RT plus concomitant TMZ and adjuvant TMZ (18 vs. 17.4 months, $P = 0.43$). The same result is achieved by using a Wilcoxon (Breslow) test for the equality of survivor functions. The Kaplan–Meier actuarial survival curves are shown in Fig. 2. Multivariate analysis revealed no difference in survival between the two trials, determined separately for any subgroup considered.

In the historical control group of 29 patients treated with RT alone after surgery, median OS was 10 months (95% CI, range 8.7–12.8 months), with an OS at 6, 12, 18 and 24 months of 78, 35, 11 and 4%, respectively. From the comparison with the group of 64 patients of both protocols, the survival benefit for patients treated with TMZ is statistically significant ($P = 0.0009$).

Discussion

Several findings demonstrate the growing role of chemotherapy in the first-line treatment of GBM. Meta-analyses [3,4], randomized trials comparing RT alone after neurosurgery with RT plus chemotherapy [19,20] and randomized trials without an RT-only arm [26] show that chemotherapy provides increase in survival. This is what we can describe, with DeAngelis [27], as a 'new beginning'. The protocol of the EORTC 26981/22981–NCIC CE3 intergroup trial, with TMZ concomitant and sequential to RT [19], will likely be considered the new standard of care and a term of comparison for future studies; this is because the study definitively compared RT alone with RT and chemotherapy by an admirable methodology, in both qualitative and quantitative terms [28], with an excellent compliance of the 85 participating centers to protocol guidelines (573 patients randomized). The study by Athanassiou *et al.* [20] confirms the advantage of a concomitant and sequential administration

of TMZ, compared with RT alone, although with a slightly different adjuvant schedule.

Our non-randomized study, on a large group of 64 patients with newly diagnosed GBM, concurs that administration of TMZ in first-line treatment with neurosurgery and RT is effective (median survival of 17.4 months, 2-year survival rate of 24%). Compared with a historical well-matched control group, survival appears to be significantly better than that with RT alone (median survival of 10 months, 2-year survival rate of 4%, $P = 0.0009$). The safety of TMZ was confirmed to be excellent, in both a combination with RT and an adjuvant phase: no incidence of grade 3–4 neutropenia was reported in the concomitant phase and the incidence of grade 3–4 neutropenia was 5–7% in the adjuvant phase; the incidence of grade 3–4 thrombocytopenia was 7% in the concomitant phase and 7–10% in the adjuvant phase. Although in the concomitant phase 30% of patients developed grade 3–4 lymphocytopenia, no clinical sequelae occurred.

We are now facing some new challenges to identify the optimal treatment: is the gain in survival a result only of the adjuvant phase of TMZ or does the continuous daily administration of TMZ during RT contribute? Do six cycles of adjuvant TMZ achieve the maximum gain? Is it possible and useful to identify patients who will benefit from TMZ?

The combination of TMZ and radiation was investigated *in vitro* by Wedge *et al.* [15] and Trog *et al.* [16], who showed an additive effect in two different GBM cell lines; van Rijn *et al.* [17] demonstrated an additive effect or a potentiated cell killing, depending on cell line. To date, no preclinical study has been conducted *in vivo* to investigate simultaneous exposure to radiation and TMZ. The EORTC 26981/22981–NCIC CE3 intergroup trial [19] is a randomized two-arm phase III trial, comparing RT alone with RT concomitant to TMZ followed by adjuvant TMZ. Results of that trial demonstrate that concomitant and adjuvant TMZ improves progression-free survival (median 6.9 vs. 5.0 months) and OS (median 14.6 vs. 12.1 months, 2-year survival 26.5 vs. 10.4%). Although their data, and also data from the study by Athanassiou *et al.* [20], definitively show that chemotherapy (and TMZ in particular) has a clear role in the treatment of GBM, no evidence shows that the prolonged survival is a result of the continuous daily administration of TMZ during RT plus the adjuvant phase and not only of the adjuvant treatment with TMZ. Results of preclinical studies (which, on the other hand, are not unequivocal) cannot be routinely expected in patients, without in-vivo tests or a three-arm clinical trial or a direct comparison between RT alone plus adjuvant TMZ and RT plus concomitant/adjuvant TMZ.

The present work certainly has some limitations: the non-randomized character of the two trials and the limited number of patients of the first trial (21 patients), compared with the second trial (43 patients). Nevertheless, it is the first study evaluating the role of concomitant continuous TMZ with RT, comparing two prospective phase II trials. The two trials were conducted at the same institutions with identical eligibility criteria, well-matched patients' characteristics, and the same cranial RT and adjuvant TMZ, but they differed in the absence (trial 1), or presence (trial 2), of a concomitant treatment with TMZ during RT. Our results show that the concomitant use of TMZ with RT could not demonstrate any improvement in survival when compared with RT alone: OS was similar, without statistically significant differences between patients treated with RT alone plus sequential TMZ and patients treated with RT plus concomitant and sequential TMZ (median survival of 18 vs. 17.4 months, $P = 0.43$). OS was similar at every time considered (at 6, 12, 18 and 24 months was 90 vs. 88, 71 vs. 67, 47 vs. 44 and 28 vs. 20%, respectively) and in every subgroup of patients considered.

Survival of the entire population of 64 patients of our study was relatively longer than the EORTC/NCIC arm treated with TMZ, resulting in a median OS of 17.4 months (vs. 14.6 months) and in an OS at 6, 12 and 18 months of 89, 69 and 45% (vs. 86, 61 and 39%); OS at 24 months was of 24% (vs. 26.5%). Three factors probably account for the difference. First, we considered an earlier time of study entry to calculate survival (time of surgical procedure instead of time of beginning RT, 5 weeks later, median). Second, population characteristics are different, with fewer patients less than 50 years old in our work but more patients with a good performance status and a complete resection. Third, patients of our study received a longer administration of TMZ in the adjuvant period, until progression instead of six cycles (five median cycles in our work vs. three median cycles in the EORTC/NCIC study). This finding could probably suggest a benefit in prolonging adjuvant chemotherapy with TMZ, but future and larger studies are needed to indicate a clear strategy (until progression, 12 cycles, more, other schedules).

In the present work, when analyzing survival in the context of the stratification factors considered (age, performance status, tumor's dimension, type of surgery), administration of TMZ concomitantly to RT could not demonstrate, once again, any improvement. To date, one of the most important identified prognostic factors, regarding the benefit of chemotherapy with TMZ, is the methylated status of the AGAT gene promoter. Among 206 patients of the randomized EORTC/NCIC trial, Hegi *et al.* [29] have shown that patients with a methylated AGAT (inactivated) treated with TMZ concomitant and sequential to RT had a longer survival

(median survival of 21.7 months). More studies are needed to demonstrate in which phase of the treatment (concomitant, sequential or both) administration of TMZ is indicated by the methylated status of the AGAT gene promoter.

In conclusion, the present work confirms that TMZ is effective and well tolerated as a first-line chemotherapeutic agent in GBM, in association with RT. Nevertheless, results of our study show that TMZ does not seem to be a radiosensitizer and a concomitant use of the drug during RT does not seem to improve survival, although it does not increase hematologic toxicity. In consideration of the aforesaid limitations of our study, a larger and randomized phase III trial should be performed to definitely confirm our findings.

The verified safety of the drug can prompt one to use it for a longer adjuvant period, as we did, prolonging for more than the six standard cycles.

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