Recurrence Pattern After Temozolomide Concomitant With and Adjuvant to Radiotherapy in Newly Diagnosed Patients With Glioblastoma: Correlation With MGMT Promoter Methylation Status

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ABSTRACT

Purpose
The aim of the present study was to evaluate factors predicting the recurrence pattern after the administration of temozolomide (TMZ), initially concurrent with radiotherapy (RT) and subsequently as maintenance therapy, which has become standard treatment for patients with newly diagnosed glioblastoma (GBM).

Patients and Methods
Ninety-five patients with newly diagnosed GBM were treated with RT plus TMZ (75 mg/m²/d) followed by maintenance TMZ cycles (150 to 200 mg/m² for 5 days every 28 days). Assessable MGMT methylation status and magnetic resonance imaging follow-up were mandatory in all cases.

Results
After a median follow-up of 18.9 months (range, 6.6 to 44.8 months), 79 patients (83%) had recurrence: inside the RT field in 57 patients (72.2%), outside in 17 patients (21.5%), and at RT margin in five patients (6.3%). MGMT status was correlated with the site of recurrence, which occurred inside, or at the margin of, the RT field in 51 patients (85%) with MGMT unmethylated status and in 11 patients (57.9%) with MGMT methylated status (P = .01). Recurrences outside the RT field occurred after a longer time interval than those inside the RT field (14.9 v 9.2 months, P = .02).

Conclusion
After the administration of TMZ concomitant with and adjuvant to RT in patients with GBM, the pattern of, and time to, recurrence are strictly correlated with MGMT methylation status.

INTRODUCTION

Postsurgical radiotherapy (RT) has been the mainstay in the treatment of newly diagnosed glioblastoma (GBM). Yet, despite the higher RT dosage currently administered and the availability of three-dimensional (3D) RT field conformation, local recurrences occur in the vast majority of patients, whereas recurrences outside the RT field are relatively rare.1-5

Data recently reported in the randomized European Organisation for Research and Treatment of Cancer 22981/26981–National Cancer Institute of Canada CE.3 (EORTC/NCIC) phase III trial,6 in which newly diagnosed patients with GBM were given temozolomide (TMZ) plus RT, have led to a new standard of care for these patients. Moreover, in the companion study by Hegi et al,7 O6-methylguanine DNA methyltransferase (MGMT) promoter methylation status was found to be a potent prognostic factor in this patient category. However, although improvements have been made in the treatment of GBM and our knowledge of genetics, the vast majority of patients treated for this type of cancer are still at risk of recurrence. Efforts must therefore be made to further investigate any correlations between recurrences, molecular genetics, and a new therapeutic standard. The study was therefore conducted to analyze the pattern of recurrence in newly diagnosed patients with GBM prospectively treated with prolonged maintenance therapy. All patients analyzed in the present study had assessable MGMT promoter methylation status and underwent magnetic resonance imaging (MRI) at the time of recurrence.
Patient Eligibility

A prospective enrollment was made of adult patients with newly diagnosed GBM who underwent surgery and had WHO performance status ≤ 2, age ≥ 18 years and less than 70 years, neutrophils ≥ 1.5 × 10^9 cells/L, platelets ≥ 100 × 10^9 cells/L, bilirubin less than 1.5× the upper limit of the normal range, alkaline phosphatase and transaminases less than 2.5× the upper limit of the normal range, serum creatinine ≤ 150 mmol/L (< 1.7 mg/dL), assessable MGMT promoter methylation status, availability of MRI imaging at the time of recurrence, and effective contraception (patients with childbearing potential). Pregnant or breast-feeding patients were excluded from the study, as were patients who had previously received cytotoxic therapy, presented active infection or other uncontrolled diseases, had psychiatric disturbances, and/or had a history of cancer other than resected nonmelanoma skin cancer or carcinoma in situ of the uterine cervix. All patients signed a form giving their fully informed consent to participate in the study, which was approved by the institutional review boards of Padova and conducted according to the principles of the Declaration of Helsinki and the rules of Good Clinical Practice.

Treatment Plan

RT. RT consisted of a conventionally fractionated regimen, with the delivery of a total dose of 60 Gy in 6 weeks, in a once-daily schedule of 2 Gy per fraction for a total of 30 fractions. Patients were treated using megavoltage equipment, such as linear accelerator beams with minimal nominal energy of 6 MV. For simulation, performed using a clinical computed tomography (CT) simulator and a helical image acquisition technique, all patients were immobilized using a commercially available thermoplastic mask system. CT image data, reconstructed in 2.5- or 3-mm slice thicknesses, were coregistered with available MRI data in T2 or fluid attenuation inversion recovery and T1 postcontrast weighting. The gross tumor volume (GTV) consisted of the entire visible tumor at preoperative contrast-enhanced CT or MRI. The clinical target volume (CTV) included the entire enhanced tumor (according to preoperative contrast-enhanced CT or MRI). Only one volume was considered throughout RT, and no cone down or boost volume was foreseen. Organs at risk, such as the eyes, optic nerves, optic chiasm, and brainstem, were delineated, and great care was taken to limit the dose to the optic chiasm and brainstem to less than 55 Gy and the retina to less than 50 Gy and to ensure that the lens was not encompassed by any direct beam. The exact gross target volume GTV-CTV margin was left to the best estimate of each individual investigator, being dependent on factors such as tumor location, amount of edema, and previous use of corticosteroids. In cases of apparently complete or subtotal removal, the position of the tumor bed was shifted, and CTV estimation took pre- and postoperative CT/MRI into account. In cases of complete surgical removal, care was taken to ensure that the GTV-CTV margin was still 2 to 3 cm, and this measurement was left to the discretion of individual radiation oncologists. The margin between CTV–planned tumor volume did not exceed 0.5 cm.

The techniques used for 3D-CRT varied slightly with each prescription. The predominant method for 3D-CRT delivery was a three-field technique (anterior-posterior and posterior-anterior field arrangement with a lateral oblique field) using 6-MV photons with custom blocking or a multileaf collimator. Treatment planning and dose calculation were based on reports 50 and 62 of the International Commission on Radiation Units and Measurements, and a dose volume histogram was mandatory. Most patients were treated while supine, but alternative appropriate positions were allowed whenever necessary.

Chemotherapy. Patients were treated with RT plus continuous daily TMZ (75 mg/m^2/d) followed by 12 cycles of maintenance TMZ (150 to 200 mg/m^2) for 5 days every 28 days; treatment was suspended after 12 cycles only if the MRI showed no enhancement suggesting presence of tumor; otherwise, chemotherapy was delivered until complete response or clear disease progression (PD). The first MRI was planned 4 weeks after the end of concurrent chemoradiation. Patients with stable disease and those with no evident lesion were considered as having non-PD and TMZ was continued. In cases of lesion growth, potentially because of early delayed reactions after RT and altering immediate post-RT neuroradiologic imaging, two more cycles were delivered, followed by another MRI. At this point, the lesions were considered pseudo PD if they were stable or had improved; otherwise they were registered as early PD and TMZ was suspended. TMZ was suspended any time the MRI evidence of a new lesion outside the RT field. All patients with pseudo PD and non-PD could experience PD in the course of the disease. Moreover, patients were evaluated taking into account clinical and neuroradiologic examinations (performed monthly before each cycle) according to MacDonald’s criteria. Neurologic status was assessed by considering signs and symptoms that, with respect to the previous examination, were possibly correlated with PD; each variation in daily corticosteroids dosage was recorded.

MGMT Status Assessment

MGMT status was evaluated by means of the methylation-specific polymerase chain reaction following a nested-polymerase chain reaction protocol,16 using methods and assessment criteria described elsewhere. The results obtained were verified using a second step for nested polymerase chain reaction; the entire process was repeated in triplicate in some cases.

Analysis of Recurrence Pattern

All recurrence patterns were analyzed by an independent review team consisting of a neuroradiologist, a radiotherapist, and an oncologist, and the members of this team were blinded to MGMT methylation status. Recurrences were defined as “in field” if ≥ 80% of the tumor recurrence resided within the prescription 95% isodose surface, and “marginal” if 20% to 80% of the lesion was inside the 95% isodose surface. In all other cases, recurrences were defined as outside the radiation field.

Statistical Analysis

Tumor PD was defined, according to MacDonald’s criteria,9 as a 25% increase in tumor size, the appearance of new lesions, or an increased need for corticosteroids. Time to PD and overall survival, measured from the time of surgery to PD or death, respectively, or date of last follow-up, were analyzed using the Kaplan–Meier method; 95% CIs were calculated using the associated estimated SEs. The log-rank test was used to test the significance of the following prognostic variables: MGMT promoter methylation status, age, sex, extent of surgery, performance status, and pattern of disease recurrence. In view of their small number, patients with recurrence at RT margin were not included in the analysis of time to PD. Multivariate analysis was performed using the Cox proportional hazards model. Significance was set at P < .05.

RESULTS

Between September 2001 and January 2007, 208 patients with newly diagnosed GBM were treated with concurrent RT/TMZ followed by 12 cycles of maintenance chemotherapy according to the above described protocol. MGMT promoter methylation status was assessable in 103 patients, for 95 of whom MRI images of recurrence were also available. The study was therefore performed on 95 patients. The patients’ baseline characteristics are listed in Table 1.

At a median follow-up of 18.9 months (range, 6.6 to 44.8 months), 79 patients (83.2%) had recurrences, which occurred more frequently in patients with MGMT unmethylated status (60 of 63 patients, 95.2%) than in those with MGMT methylated status (19 of 32 patients, 59.4%; P = .0001).

Pattern of Recurrence

Recurrence occurred inside the RT field in 57 patients (72.2%), outside in 17 patients (21.5%), and at RT margin in five patients (6.3%); examples of recurrences outside the RT field are shown in...
No patients had CSF/spinal or distant disease recurrence. The median number of maintenance TMZ cycles was five (range, one to 18 cycles) for patients with recurrence inside the RT field, five (range, one to 12 cycles) for patients with recurrence at RT margin, and 11 (range, one to 15 cycles) for patients with recurrence outside the RT field. Recurrence occurred inside the RT field and at the margin of the RT field in 51 patients (85%) with MGMT unmethylated status and in 11 patients (57.9%) with MGMT methylated status (\( P = .01 \)), respectively.

PD inside the RT field occurred more frequently in patients who underwent subtotal surgical excision than in those who underwent complete resection (65.8% vs 90.2%, \( P = .009 \)).

**Outcome**

The median time to PD was 9.2 months (range, 8.2 to 10.1 months) in patients with recurrence inside, and 14.9 months (range, 11.2 to 19.7 months) in patients with recurrence outside the RT field (\( P = .02 \)), as shown in Figure 2.

The median survival was 17.3 months (range, 15.1 to 20 months), 14.8 months (range, 10.8 months to not reached), and 26.1 months (range, 19 months to not reached) in patients with recurrence inside, at the margin, and outside the RT field, respectively. As shown in Figure 3, the survival rate was significantly higher in patients with recurrence outside than those with recurrence inside the RT field (\( P = .013 \)).

**DISCUSSION**

Data from the EORTC/NCIC phase III trial, which have generated a new standard of care in the treatment of newly diagnosed patients with GBM, represent a milestone after more than two decades of ineffective efforts at prolonging the survival of this patient category. Before this new mainstay treatment was established, RT with or without adjuvant chemotherapy, considered standard treatment, yielded a median overall survival of approximately 1 year and a 2-year overall survival rate of 8%. Interestingly, the addition of TMZ concomitant to RT, and later, as maintenance treatment, has improved not only the median overall survival, but more importantly, the 2-year

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Two examples for comparison between radiotherapy plan and magnetic resonance imaging at time of recurrence. (A) Disease before surgical excision. (B) Radiotherapy plan. (C) Recurrence outside the radiotherapy field.
overall survival rate. Further information provided by the EORTC/NCIC study threw important light on the role of MGMT promoter methylation status as a potent prognostic factor.

However, despite advances made in the treatment of GBM and the improved knowledge of genetics, the vast majority of patients with GBM are still at risk of recurrence. In the past, recurrences after RT were mainly local. Data, reported by Hochberg and Pruitt in 1980 and Wallner et al in 1989, on patients with high-grade glioma treated with whole-brain RT showed that approximately 80% of tumors recurred within 2 cm of the initial tumor bed and that only 3% to 5% of failures occurred outside this margin, thus indicating that the addition of whole-brain RT to localized treatment was probably unnecessary, given the local failure patterns observed. Subsequent studies using CT-based treatment planning, delivering 60 to 66 Gy (target tumor, 2- to 3-cm margin), confirmed that the pattern location of initial failure shifted from local/marginal to distant, recurrences occurring within 2 cm of the initial enhancing area (peripheral) in 25 patients (79%) and beyond the 2-cm margin (distant) in five patients (17%); in one patient (3%), recurrences were peripheral and distant.

To our knowledge, the present study could be the first in literature to evaluate the recurrence pattern after the administration of TMZ as a radiosensitizing agent concurrent with RT and, as a cytotoxic drug, in the maintenance setting. Because of strict inclusion criteria, there may have been the possibility that a selection bias exists, and the generalizability is therefore applicable only to patients who are ≤ 70 years of age and who undergo resection. We observed a failure pattern shift from local/marginal to distant, recurrences outside the RT field occurring in approximately 20% of cases. Furthermore, distant recurrence occurred significantly later than local recurrence, and the recurrence pattern was significantly influenced by MGMT methylation status, recurrences being outside the RT field in 15% and 42% of patients with MGMT unmethylated and MGMT methylated status, respectively (P = .01). Although the factors underlying this phenomenon are not clear, some considerations should be made. It is unclear whether MGMT methylation status alters the motility and migration pattern of GBM cells, or whether the combined chemoradiation approach, by having a synergic effect on MGMT methylated cells, can enhance local GBM cell eradication. The latter hypothesis is supported by the finding that the time to recurrence was prolonged in patients with MGMT methylated status, as was the time to distant recurrence (14.9 months versus 9.2 months in patients with local failure). Interestingly, in the present study, after a median follow-up of 18.9 months, 16 patients have not yet had disease progression; moreover, three of these 60 patients (5%) had MGMT unmethylated status and 13 (22.4%) had MGMT methylated status.

Because MGMT assessment, an inclusion criterion in the present study, is more frequently available in larger surgical tumor samples, it is highly likely that our cohort consisted of patients with more advantageous prognostic factors (ie, extent of surgery). Moreover, even if this feature was well balanced between MGMT methylated and unmethylated status, a prospective trial on a larger patient cohort should be conducted to validate our findings. The findings made in patients with GBM do show that in patients with MGMT unmethylated status, a greater effort must be made to improve local control, and new agents should be evaluated for use in combination with RT or RT/TMZ. In patients with MGMT methylated status, the addition of TMZ to RT is effective in controlling GBM cells in the tumor bed, but not in controlling distant recurrence.

The discovery that the tumoral bed was the main site of recurrence led to further attempts to improve on local treatments on the basis of the hypothesis that an increased dosage might be required to eradicate high-grade astrocytomas. Nakagawa et al, who treated patients with GBM with conformal postoperative RT in doses ranging from less than 60 to 90 Gy, found that the incidence of distant failures in the 90-Gy arm was significantly higher than that in the less-than-90-Gy arm (69% versus 16%, respectively).

Studies using radiosurgery and interstitial implants have demonstrated an increased proportion of failures outside the high-dose field. In their study on 31 patients with GBM treated with external-beam RT (median dose, 54 Gy/1.8-Gy fractions) plus radiosurgery (median maximum dose, 18.6 Gy), Mehta et al found that no patients had failures within the high-dose region, whereas recurrences occurred within 2 cm of the initial enhancing area (peripheral) in 25 patients (79%) and beyond the 2-cm margin (distant) in five patients (17%); in one patient (3%), recurrences were peripheral and distant.

Overall survival by pattern of recurrence. Continuous line in blue indicates patients with recurrences outside the radiation field; continuous line in yellow indicates patients with recurrences inside the radiation field. RT, radiotherapy.
A further open question is whether the so-called distant recurrence of the primary tumor should be considered true recurrence or, in view of the prolonged survival of these patients, a second tumor occurring as a result of the presence of predisposed tissue or to genetic or environmental factors, as in, for example, head and neck tumors. Moreover, on account of the high chemosensitivity of tumors cells harboring the methylated MGMT gene promoter, protracted maintenance TMZ treatment, beyond the six cycles used in the EORTC/NCIC trial, might allow a prolonged control of disease, which would no longer be considered local, but involving the entire brain.

It is evident that extensive-field RT is inappropriate for the above patient category, in view of their prolonged survival and the related RT field and the risk of cognitive impairment. In the future, however, the definition of GTV and CTV may be achieved with the use of MRI and fusion-imaging programs, as the higher incidence of recurrence outside the RT field may depend on the inadequate identification of the target with standard CT scan. The use of spectroscopy in establishing the limit for GTV and CTV may be helpful in overcoming this serious disadvantage.