MGMT Promoter Methylation Status Can Predict the Incidence and Outcome of Pseudoprogression After Concomitant Radiochemotherapy in Newly Diagnosed Glioblastoma Patients

Alba A. Brandes, Enrico Franceschi, Alicia Tosoni, Valeria Blatt, Annalisa Pession, Giovanni Tallini, Roberta Bertorelle, Stefania Bartolini, Fabio Calliucci, Alvaro Andreoli, Giampiero Frezza, Marco Leonardi, Federica Spagnolli, and Mario Ermani

ABSTRACT

Purpose

Standard therapy for glioblastoma (GBM) is temozolomide (TMZ) administration, initially concurrent with radiotherapy (RT), and subsequently as maintenance therapy. The radiologic images obtained in this setting can be difficult to interpret since they may show radiation-induced pseudoprogression (psPD) rather than disease progression.

Methods

Patients with histologically confirmed GBM underwent radiotherapy plus continuous daily temozolomide (75 mg/m²/d), followed by 12 maintenance temozolomide cycles (150 to 200 mg/m² for 5 days every 28 days) if magnetic resonance imaging (MRI) showed no enhancement suggesting a tumor; otherwise, chemotherapy was delivered until complete response or unequivocal progression. The first MRI scan was performed 1 month after completing combined chemoradiotherapy.

Results

In 103 patients (mean age, 52 years [range 20 to 73 years]), total resection, subtotal resection, and biopsy were obtained in 51, 51, and 1 cases, respectively. MGMT promoter was methylated in 36 patients (35%) and unmethylated in 67 patients (65%). Lesion enlargement, evidenced at the first MRI scan in 50 of 103 patients, was subsequently classified as psPD in 32 patients and early disease progression in 18 patients. PsPD was recorded in 21 (91%) of 23 methylated patients (35%) and unmethylated in 67 patients (65%). Improvement in the early recognition of psPD patterns and knowledge of mechanisms underlying this phenomenon are crucial to eliminating biases in evaluating the results of clinical trials and guaranteeing effective treatment.

Conclusion

PsPD has a clinical impact on chemotherapy-treated GBM, as it may express the glioma killing effects of treatment and is significantly correlated with MGMT status. Improvement in the early recognition of psPD patterns and knowledge of mechanisms underlying this phenomenon are crucial to eliminating biases in evaluating the results of clinical trials and guaranteeing effective treatment.

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INTRODUCTION

Data recently reported in the randomized EORTC 22981/26981–NCIC CE.3 (European Organisation for Research and Treatment of Cancer/National Cancer Institute of Canada) phase III trial on newly diagnosed patients with glioblastoma (GBM) patients’ given temozolomide (TMZ) plus radiotherapy (RT) have provided a new standard of care. A small, but significant, progression-free survival (PFS) advantage (5 months v 6.9 months) has been achieved with this approach, whereas a marked and significant benefit has been obtained in 2-year overall survival (11% v 27%). This type of effect is not frequent in medical oncology, where significant PFS advantages do not often translate into an overall survival advantage. The conversion of a small PFS advantage into a consistent survival benefit may depend on the overestimation of disease progression in the temozolomide-radiotherapy arm. Classically, response evaluation in neuro-oncology is based on planimetric variations in enhanced lesions, but it is also based on corticosteroids dosage and variations in neurological conditions. However, the brief time...
interval from the end of radiotherapy and its combination with a sensitizing agent such as TMZ could create biases in neuroradiological imaging evaluation. Radiation injury to the CNS may, in fact, depend on increased capillary permeability induced by radiotherapy, leading to fluid transudation into the interstitial space and consequent brain edema. Furthermore, if capillary permeability is altered, damage from chemotherapy may occur earlier and be more severe; radiotherapy may enhance the efficacy of chemotherapy by maximizing drug uptake either at the cell membrane, through a disruption of the blood-brain barrier, and/or through an alteration in cell metabolism.3-5 This can lead to the observation of an early radiological increase in contrast enhancement at magnetic resonance imaging (MRI) consequent to alterations in the blood-brain barrier, thus falsely suggesting tumor progression. This phenomenon (also called therapy-induced necrosis or pseudoprogression [psPD], which may be the expression of treatment–induced necrosis) leads to the rupture of the hematoencephalic barrier and the passage of contrast medium. Although this phenomenon has long been known,3,4,6 its real incidence has not yet been reported in a large series of patients given concomitant radiotherapy and TMZ treatment; nor has the potential impact of O6 alkylating agents on the blood–brain barrier been reported.7-9 This may be the expression of increased blood flow and blood–brain barrier permeability in the tumor bed, due to antiangiogenic activity of TMZ.10-11 Therapy-induced necrosis (also called therapy-induced necrosis) is a common observation in glioblastoma patients treated with radiotherapy plus continuous daily temozolomide (75 mg/m²/d), followed by 12 maintenance temozolomide cycles (150 to 200 mg/m² for 5 days every 28 days).

A retrospective analysis was made to correlate MGMT promoter methylation status and type of progression. All patients signed a form giving their fully informed consent to take part in the prospective study on the duration of maintenance chemotherapy; they also gave their consent in writing for research tests to be conducted on the tissue blocks obtained from them in any future research projects approved by ethical committee and aiming to improve on the understanding and treatment of brain tumors. The study, approved by the institutional review board of Padova Azienda Ospedaliera (Padova, Italy), was conducted according to the principles of the Declaration of Helsinki and the rules of Good Clinical Practice.

**MGMT Status Assessment**

MGMT status was evaluated with the methylation specific polymerase chain reaction (MSP) after a nested-polymerase chain reaction protocol,6 using methods and assessment criteria described elsewhere. Because the quality of DNA obtained from formalin-fixed, paraffin-embedded tumor tissue affects the success rate of MSP, in some cases MGMT methylation status was determined using a different nested MSP approach, with a first pair of primers to obtain smaller amplicons (129 base pairs), for which forward and reverse primers have been described.4,6 The results obtained in the present study were verified using a second step of both modification and nested polymerase chain reaction; the entire process was repeated in triplicate in some cases.

**Statistical Analysis**

Tumor progression was defined according to MacDonald’s Criteria2 as a 25% increase in tumor size, the appearance of new lesions, or an increased need of corticosteroids. Time-to-progression (TTP) and overall survival (OS) were measured from the time of surgery to disease progression or death, respectively, or date of last follow-up, and analyzed using the Kaplan-Meier method: 95% CIs were calculated using the associated estimated SEs. The log-rank test was employed to compare MGMT promoter methylation status, methylated versus unmethylated and psPD versus ePD and to test the significance of the following prognostic variables: age, extent of surgery, and performance status.11 Multivariate analysis was performed using the Cox proportional hazards model. Significance level 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. An analysis was made of all patients (n = 103) for whom MGMT promoter methylation status was assessable. The median follow-up of patients included in the analysis was 18.93 months (range, 6.6 to 62 months). The patients’ baseline characteristics are presented in Table 1. The median number of maintenance TMZ cycles was 6 (range, 0 to 30 cycles). One patient had rapid disease progression after completion of concomitant treatment, and it was impossible to administer the first cycle of maintenance chemotherapy; this patient was therefore considered ePD, and his data was included in the analysis.

**Toxicity**

During the concomitant therapy phase, grade 4 neutropenia occurred in one patient (1%), and grade 3 to 4 thrombocytopenia in four patients (3.9%). Grade 1 to 2 lymphocytopenia occurred in 10 patients (9.7%). One patient had pneumonia with normal WBCs. During the maintenance therapy phase, grade 3 to 4 neutropenia and thrombocytopenia occurred in 2% and 5% of patients, respectively. Two patients discontinued treatment in the maintenance phase of therapy: one during the third cycle due to prolonged grade 4 thrombocytopenia, and one after the fifth cycle due to prolonged grade 2 thrombocytopenia.

**METHODS**

**Patient Eligibility**

Adult patients with newly diagnosed GBM were prospectively enrolled onto the trial if they had a WHO performance status of 2 or less and adequate hematological, renal, and hepatic function (absolute neutrophil count, $\geq 1,500/\text{mm}^3$; platelet count, $\geq 100,000/\text{mm}^3$; serum creatinine level, $\leq 1.5$ times the upper limit of the normal laboratory range; total serum bilirubin level, $\leq 1.5$ times the upper limit of the normal range; liver-function values, $< 3$ times the upper limit of the normal laboratory range). Patients were treated with radiotherapy plus continuous daily temozolomide (75 mg/m²/d) followed by 12 cycles of maintenance temozolomide (150 to 200 mg/m² 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. The first MRI was planned 4 weeks after the end of concurrent chemoradiotherapy. If patients presented stable disease or had no evident lesion, they were considered as having nonprogressive disease (non-PD) and TMZ was continued. In cases of lesion growth, which may be due to potential early-delayed reactions after RT,7 altering immediate post-RT neuroradiological imaging, another two cycles were delivered followed by another MRI. At this point, the lesions were considered psPD if they were stable or had improved; otherwise they were registered as early disease progression (ePD) and TMZ was suspended. TMZ was suspended at anytime if the MRI image evidenced a new lesion outside the radiotherapy field. All patients with psPD and non-PD could experience a PD after RT,7 altering immediate post-RT neuroradiological imaging, another two cycles were delivered followed by another MRI. At this point, the lesions were considered psPD if they were stable or had improved; otherwise they were registered as early disease progression (ePD) and TMZ was suspended. TMZ was suspended at anytime if the MRI image evidenced a new lesion outside the radiotherapy field. All patients with psPD and non-PD could experience a PD in the course of the disease. Moreover, patients were evaluated taking into account clinical and neurological examinations (performed monthly before each cycle) according to MacDonald’s criteria7 by a multidisciplinary team consisting of an oncologist and a neuroradiologist. Neurological status was assessed by considering signs and symptoms possibly correlated with progress with respect to the previous examination; each variation in daily corticosteroids dosage was recorded.

MGMT and Pseudoprogression After Concomitant Radiochemotherapy in Glioblastoma
Evaluation at First MRI After Concomitant Radiochemotherapy and Correlation With MGMT Status

At the first MRI scan, performed 1 month after concurrent RT/TMZ, lesion enlargement was recorded in 50 patients (48.5%), while 53 patients were non-PD. The findings were psPDs in 32 (64%) of 50 patients and ePDs in 18 (36%) of 50 patients. MGMT promoter was methylated in 21 (66%) of the 32 psPD patients and in two (11%) of the 18 ePD patients ($P = .0002$). Thirteen (25%) of the 53 non-PD patients had MGMT promoter methylated and the other 40 patients had MGMT promoter unmethylated status (Fig 1). A significant difference was found between the non-PD and the psPD group ($P = .0002$), but not between non-PD and ePD groups ($P = .23$), for MGMT promoter status. Clinical deterioration was found in 21 (42%) of 50 patients with enlarged lesion images, being present in 10 (55.6%) of 18 with ePD, and in 11 (34%) of 32 patients with psPD ($P = .14$). All patients with psPD and clinical deterioration had a recovery of clinical function at a median time of 7 months (range 1.2 to 18 months). MGMT promoter status predicted psPD in 91.3% of methylated cases (95% CI, 72% to 99%), but predicted ePD in only 59% of unmethylated cases (95% CI, 38% to 76%).

TTP

In the present study, MGMT status significantly influenced overall median TTP, which was 11.7 months (95% CI, 8.9 to 14.5 months), being 21.9 months (95% CI, 12.9 to 30.8 months) in MGMT promoter methylated patients and 9.2 months (95% CI, 8.5 to 9.8 months) in MGMT promoter unmethylated patients ($P < .0001$). Extent of surgery ($P = .44$), age ($P = .69$) and performance status ($P = .86$) were not significantly correlated with TTP. In 85 patients (32 psPD; 53 non-PD), the psPD patients had a significantly longer mTTP than the non-PD patients ($P = .0001$; Table 2). In the subgroup of patients with psPD, the median time interval between recording psPD and subsequent real progression was 16.2 months: 21 months in MGMT promoter methylated patients and 15.3 months in MGMT promoter unmethylated patients ($P = .41$). Subsequent disease progression was recorded in 21 (65.6%) psPD and in 46 (86.8%) non-PD patients ($P = .02$).

Overall Survival

A median survival of 20.7 months was achieved (95% CI, 17.3 to 24 months): 43.6 months (95% CI, 25.5 to 61.7 months) and 16.8 months (95% CI, 14.1 to 19.6 months) in methylated MGMT promoter and in unmethylated MGMT promoter patients, respectively ($P < .0001$; Fig 2). In 53 patients without images of lesion increase after combined chemoradiotherapy, censored patients were significantly higher in methylated MGMT promoter subgroup (nine of 13 patients; 69%) with respect to the unmethylated MGMT promoter subgroup (nine of 40 patients; 22.5%; $P = .002$) despite the median follow-up for methylated MGMT promoter being significantly higher than in unmethylated MGMT promoter patients (21.6 and 17.8

![Table 1. Baseline Characteristics of Patients](image)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients</th>
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<tbody>
<tr>
<td>No. %</td>
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<tr>
<td>Age, years</td>
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<td>Median</td>
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<tr>
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Abbreviation: MGMT, O6-methylguanine-DNA methyltransferase.
multivariate analysis, survival was significantly influenced by promoter methylation status (surgery (was not evaluated in the regression model.

De Wit et al,13 who showed that transient neuroradiological enhancement sim-
ulating progression can appear within 3 months after the end of radiotherapy, focused on the potential risk of including patients in clinical trials on recurrent disease that is not really in progression but in psPD.

Chamberlain et al12 evaluated 65 GBM patients treated with concurrent RT and TMZ and reported that seven of 15 (46%) of those who underwent surgical resection for suspected recurrence had historically confirmed psPD with patterns of radiation-induced necrosis.

Using proton MR spectroscopic imaging, specific changes were reported in cases of radiation injury including a reduction in N-acetylaspartate (NAA) and various changes in choline and creatine levels and/or alterations in choline/NAA and choline/creatine ratios, described elsewhere.16,17 Moreover, perfusion MRI is considered a useful tool in the diagnosis of recurrence and necrosis; changes in cerebral tumor blood volume occurring during the early radiotherapy course can also be predictive of survival. Some authors have observed that apparent diffusion coefficient values are useful in distinguishing between high-grade glioma and normal tissue, though they do not allow a differentiation between a high-grade glioma and the surround- ing edema.18,19 Nevertheless, to date, the only available way of distin-
guishing between psPD and PD by conducting a follow-up on patients with early enlarged images, as standard MRI is not sufficient, nor have alternative neuroradiological techniques been validated in prospective trials. Furthermore, the real impact of this entity has not yet been established due to the absence of prospective studies on large series consisting exclusively of patients who have been treated with concomitant radio-chemotherapy. The findings made in the present study show, for the first time, that the real incidence of psPD in GBM patients treated with concomitant TMZ and RT is 30%. Moreover, in approximately 50% of patients, the first MRI scan images after com-
ized RT/TMZ were doubtful for progression, but only 36% of these patients were subsequently evaluated as true ePD; the other 64% had a psPD. Therefore, the next step for clinical research should be a priori identification of patients with psPD. We found that there is a 91.3% (95% CI, 72% to 99%) probability of psPDs in patients with methyl-
ated MGMT promoter tumors and a 59% (95% CI, 38% to 76%) probability of early PD in unmethylated MGMT promoter tumors. If the probability of methylated MGMT promoter patients having psPD is high, it is almost equally probable that unmethylated MGMT pro-
moter patients will have psPD or ePD if the first MRI images reveal

<table>
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<th>Characteristic</th>
<th>TTP (months)</th>
<th>OS (months)</th>
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<td>ePD</td>
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Abbreviations: MGMT, O6-methylguanine–DNA methyltransferase; TTP, time to disease progression; OS, overall survival; psPD, pseudoprogression; ePD, early disease progression; PD, disease progression. *P = significant

Fig 3. Overall survival: blue line, patients with pseudoprogression; gray line, patients with early disease progression; yellow line, patients with neither pseudoprogression nor early disease progression.

Fig 2. Overall survival by presence of O6-methylguanine–DNA methyltransferase (MGMT) promoter methylation status. Blue line, patients with methylated MGMT promoter; yellow line, patients with unmethylated MGMT promoter status.

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lesion enlargement. New prospective studies testing new neuroradiological techniques on larger patient populations are therefore required in order to obtain sounder findings, and to study alternative psPD predictors. The higher rates of methylated MGMT promoter found in patients with psPD is probably correlated with the efficacy of concurrent RT/TMZ treatment on the residual tumor burden; in this setting the neuroradiological image of psPD may represent not only a treatment-induced blood brain barrier disruption, but also reflect the efficacy of therapy, since the OS of patients with psPD is significantly higher than in those without psPD.

It has not yet been demonstrated that maintenance chemotherapy prolongs the survival of patients with solid tumors. However, prolonged TMZ therapy can substantially deplete MGMT,20 thus providing the rationale for continuous treatment. In the present study, patients who received less than six TMZ cycles had an OS of 13.7 months, while those who received \( \geq \) six TMZ cycles had an OS of 34.8 months (\( P < .0001 \)). However, in view of the presence of several factors that may have influenced the duration of maintenance therapy, we decided not to perform a multivariate analysis of this datum, also in view of the nonrandomized nature of our trial.

More information is required for a better understanding of the nature of psPD in order to distinguish it from real early PD, thus obviating biases in the evaluation of results from clinical trials, and preventing patients from being denied effective treatment. Trials should also be conducted to evaluate the predictive value of novel neuroradiological techniques, such as the impact of prolongation of maintenance TMZ and intensified schedules; the ongoing RTOG 0525/EORTC 26052-22053 trial is investigating this issue. Moreover, as vascular damage may play a role in the pathogenesis of this radiological pattern or therapy-induced effect, the evaluation of angiogenic pathways and correlations with MGMT status in GBM will be the backbone for future research.

### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

### AUTHOR CONTRIBUTIONS

**Conception and design:** Alba A. Brandes, Enrico Franceschi, Alicia Tosoni  
**Administrative support:** Alba A. Brandes, Valeria Blatt, Stefania Bartolini  
**Provision of study materials or patients:** Alba A. Brandes, Valeria Blatt, Stefania Bartolini  
**Data analysis and interpretation:** Alba A. Brandes, Enrico Franceschi, Alicia Tosoni, Valeria Blatt, Annalisa Pession, Giovanni Tallini, Roberta Bertorelle, Stefania Bartolini, Fabio Calbucci, Alvaro Andreoli, Giampiero Frezza, Marco Leonardi, Federica Spagnoli  
**Collection and assembly of data:** Alba A. Brandes, Enrico Franceschi, Valeria Blatt, Stefania Bartolini  
**Manuscript writing:** Alba A. Brandes, Enrico Franceschi, Alicia Tosoni, Marco Ermani  
**Final approval of manuscript:** Alba A. Brandes, Enrico Franceschi, Alicia Tosoni, Valeria Blatt, Annalisa Pession, Giovanni Tallini, Roberta Bertorelle, Stefania Bartolini, Fabio Calbucci, Alvaro Andreoli, Giampiero Frezza, Marco Leonardi, Federica Spagnoli, Marco Ermani

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