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 Calbucci, Alvaro Andreoli, Giampiero Frezza, Marco Leonardi, Federica Spagnoli, 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 MGMT promoter and 11 (41%) of 27 unmethylated MGMT promoter (P = .0002) patients. MGMT status (P = .001) and psPD detection (P = .045) significantly influenced survival.

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.

J Clin Oncol 26:2192-2197. © 2008 by American Society of Clinical Oncology

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–methylguanine–DNA methyltransferase (MGMT) promoter methylation status been described in this patient category. A retrospective analysis was therefore made of newly diagnosed GBM patients, with assessable MGMT promoter methylation status treated prospectively 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).

MGMT and Pseudoprogression After Concomitant Radiochemotherapy in Glioblastoma

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.6 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.8,10 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.
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 ($20.7 \pm 11.4$ months; $P = .001$; 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 months).
months, respectively; \( P < .05 \)). Extent of surgery (\( P = .10 \)), second surgery (\( P = .12 \)), age (\( P = .65 \)), and performance status (\( P = .36 \)) were not significant prognostic factors. Median survival was significantly influenced by psPD, with a value of 38 months in this group, 10.2 months in patients with ePD, and 20.2 months in patients with non-PD (\( P < .001 \); Fig 3), and by the number of TMZ cycles administered (< 6 cycles \( v = 6 \) cycles; 13.7 and 34.8 months; \( P < .0001 \)). At multivariate analysis, survival was significantly influenced by MGMT promoter methylation status (\( P < .001 \)) and by the detection of psPD (\( P = .045 \)). As the number of TMZ is not an independent variable, it was not evaluated in the regression model.

**Table 2.** Effects of MGMT Promoter Methylation Status and First MRI Findings

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TTP (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGMT promoter status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>11.7</td>
<td>20.7</td>
</tr>
<tr>
<td>Methylated</td>
<td>21.9*</td>
<td>43.6*</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>9.2</td>
<td>16.8</td>
</tr>
<tr>
<td>MRI findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>psPD</td>
<td>20.7*</td>
<td>38*</td>
</tr>
<tr>
<td>ePD</td>
<td>5.7</td>
<td>10.2</td>
</tr>
<tr>
<td>No PD images</td>
<td>11.4</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Abbreviations: MGMT, O\(^6\)-methylguanine–DNA methyltransferase; TTP, time to disease progression; OS, overall survival; psPD, pseudoprogression; ePD, early disease progression; PD, disease progression. *\( P \) = significant.

**Discussion.**

psPD was recently described in neuro-oncology as a transient blood-brain barrier alteration with neuroradiological images frequently indistinguishable from disease progression. Some authors have underlined the problems linked to this entity\(^{12-15}\) (Table 3). De Wit et al.,\(^{13}\) who showed that transient neuroradiological enhancement simulating 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 al.\(^{12}\) 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 histologically 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/creatinine 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 surrounding edema.\(^{18,19}\) Nevertheless, to date, the only available way of distinguishing 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 combined 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 methylated 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 promoter patients will have psPD or ePD if the first MRI images reveal...
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, was 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 ≥ 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.

### Table 3. Studies on psPD in GBM Patients Treated With Concurrent Chemoradiation

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Patients</th>
<th>% of psPD</th>
<th>MGMT Promoter Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamberlain et al, 200612</td>
<td>65</td>
<td>46.7*</td>
<td>Not reported</td>
</tr>
<tr>
<td>Jeffries, 200715</td>
<td>15</td>
<td>20</td>
<td>Not reported</td>
</tr>
<tr>
<td>Taal et al, 200714</td>
<td>85</td>
<td>21</td>
<td>Not reported</td>
</tr>
<tr>
<td>Present study</td>
<td>103</td>
<td>31</td>
<td>Reported</td>
</tr>
</tbody>
</table>

Abbreviations: GBM, glioblastoma; psPD, pseudoprogression; MGMT, O6-methylguanine–DNA methyltransferase.

*Calculated in patients undergoing resection for images of lesion enlargement.

### References

13. de Wit MC, de Bruin HG, Eijkenboom W, et al: Immediate post-radiotherapy changes in malignant...
glioma can mimic tumor progression. Neurology 63:535-537, 2004


