MGMT Promoter Methylation Is a Strong Prognostic Biomarker for Benefit from Dose-Intensified Temozolomide Rechallenge in Progressive Glioblastoma: The DIRECTOR Trial

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Abstract

Purpose: Rechallenge with temozolomide (TMZ) at first progression of glioblastoma after temozolomide chemoradiotherapy (TMZ/RT—TMZ) has been studied in retrospective and single-arm prospective studies, applying temozolomide continuously or using 7/14 or 21/28 days schedules. The DIRECTOR trial sought to show superiority of the 7/14 regimen.

Experimental Design: Patients with glioblastoma at first progression after TMZ/RT—TMZ and at least two maintenance temozolomide cycles were randomized to Arm A [one week on (120 mg/m² per day)/one week off] or Arm B [3 weeks on (80 mg/m² per day)/one week off]. The primary endpoint was median time-to-treatment failure (TTF) defined as progression, premature temozolomide discontinuation for toxicity, or death from any cause. O6-methylguanine DNA methyltransferase (MGMT) promoter methylation was prospectively assessed by methylation-specific PCR.

Results: Because of withdrawal of support, the trial was prematurely closed to accrual after 105 patients. There was a similar outcome in both arms for median TTF [A: 1.8 months; 95% confidence intervals (CI), 1.8–3.2 vs. B: 2.0 months; 95% CI, 1.8–3.5] and overall survival [A: 9.8 months (95% CI, 6.7–13.0) vs. B: 10.6 months (95% CI, 8.1–11.6)]. Median TTF in patients with MGMT-methylated tumors was 3.2 months (95% CI, 1.8–7.4) versus 1.8 months (95% CI, 1.8–2) in MGMT-unmethylated glioblastoma. Progression-free survival rates at 6 months (PFS-6) were 39.7% with versus 6.9% without MGMT promoter methylation.

Conclusions: Temozolomide rechallenge is a treatment option for MGMT promoter-methylated recurrent glioblastoma. Alternative strategies need to be considered for patients with progressive glioblastoma without MGMT promoter methylation. Clin Cancer Res; 21(9); 2057–64. ©2015 AACR.
Progressive or Off versus Three Weeks on One Week Off in Patients with TMZ/RT rechallenge using various regimens (9 clinical trials for recurrent glioblastoma (7, 8), temozolomide (CCNU) which has become the standard of care in randomized therapeutic approaches include nitrosoureas such as lomustine factors, and local preference, the most commonly used systemic option for patients with glioblastoma harboring MGMT promoter methylation status. Temozolomide rechallenge should no longer be considered because of second-line treatments (15, 16) and the controversy about the optimal dosing of temozolomide for patients with tumors lacking MGMT promoter methylation, but is an appropriate option for patients with glioblastoma harboring MGMT promoter methylation at first relapse.

Translational Relevance
The prospective randomized DIRECTOR trial assessed the efficacy and tolerability of two different regimens of rechallenge with intensified temozolomide (TMZ) at first progression of glioblastoma after temozolomide chemoradiotherapy (TMZ/RT—TMZ). Efficacy was similar in both arms, but depended strongly on MGMT promoter methylation status. Temozolomide rechallenge should no longer be considered for patients with tumors lacking MGMT promoter methylation, but is an appropriate option for patients with glioblastoma harboring MGMT promoter methylation at first relapse.

Introduction
The standard of care for newly diagnosed glioblastoma, with an incidence of more than 3 of 100,000 the most common primary malignant brain tumor, includes resection or biopsy as feasible, involved field radiotherapy, and concomitant and adjuvant temozolomide (TMZ/RT—TMZ; ref. 1). Although antiangiogenic agents such as the antibody to VEGF, bevacizumab, or the integrin inhibitor cilengitide failed to prolong overall survival (2–4), the novel approach of tumor-treating fields provided a survival advantage and may be incorporated into the future first-line treatment (5).

Central pathology review, DNA extraction, and MGMT promoter methylation analysis
All tissue samples from primary or recurrent tumor were confirmed by central pathology review (G. Reifenberger) to represent glioblastoma according to the World Health Organization (WHO) classification of tumors of the central nervous system (18). Tumor DNA was extracted from formalin-fixed and paraffin-embedded tissue samples using the Qiagen blood and tissue DNA extraction kit (Qiagen). Each tumor sample used for DNA extraction was histologically verified to contain viable glioblastoma tissue with an estimated tumor cell content of more than 3 of 100,000 the most common primary malignant brain tumor, includes resection or biopsy as feasible, involved field radiotherapy, and concomitant and adjuvant temozolomide (TMZ/RT—TMZ; ref. 1). Although antiangiogenic agents such as the antibody to VEGF, bevacizumab, or the integrin inhibitor cilengitide failed to prolong overall survival (2–4), the novel approach of tumor-treating fields provided a survival advantage and may be incorporated into the future first-line treatment (5).

Temozolomide rechallenge after systematic recognition of pseudoprogression as a potential confounder of second-line treatments (15, 16) and the controversy about the optimal dosing of temozolomide for patients with recurrent glioblastoma after failure of first-line TMZ/RT—TMZ led to the design of the DIRECTOR (Dose-Intensified Rechallenge with Temozolomide, One Week on One Week Off versus Three Weeks on One Week Off in Patients with Progressive or Recurrent Glioblastoma) trial, which sought to explore the activity of two widely used regimens of dose-intensive temozolomide for recurrent glioblastoma. 1 week on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (7/14; ref. 9).
Assessments and endpoints
Patients were to be seen weekly during cycle 1 and monthly thereafter for general evaluation and blood tests. Toxicity was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE v3.0). Cognitive function was assessed by Mini-Mental State Examination (MMSE) in 4 weekly intervals. Quality of life was monitored by EORTC QLQ-C30 and QLQ-BN20 in 8 weekly intervals. Disease status was monitored by MRI in 8 weekly intervals and assessed using Macdonald criteria as prespecified.

Events (CTCAE v3.0). Cognitive function was assessed by Mini-

Statistical analyses
The targeted sample size was 83 patients per arm, and no interim analysis was planned. This size would have allowed for a detection of an improvement in median TTF from 18.2 weeks for Arm B to 29.2 weeks for Arm A (HR = 0.63; refs. 10, 25). On the basis of these assumptions, there was approximately 80% power to detect the stated difference in TTF between the two treatment arms for a two-sided level of 0.05. Treatment arms were compared using a permutation test (26) with 9999 replicates in the Cox proportional hazard model with the same parameters as explanatory variables used for the treatment allocation algorithm [MGMT promoter methylation status, >2 months since previous temozolomide treatment, age at least 50 years, Karnofsky perfor-

Outcome by treatment and MGMT status
All clinical outcome parameters were comparable in Arms A and B (Fig. 2; Table 2). Median TTF was below 2 months, whereas median OS from first intake of study drug was in the range of 10 months. The P value from the permutation test (P = 0.488) was close enough to the P value using partial likelihood from the Cox model (P = 0.485) to justify taking the latter one for all other analyses. There were two CR (4%) and two PR (4%) in Arm A and four CR (8%) and four PR (8%) in Arm B by local assessment (P = 0.68) in response to the study treatment. The median duration of

Results
Patient characteristics
One hundred and five patients were randomized at 16 sites from September 2009 to June 2012. Table 1 summarizes patient characteristics per treatment arm. Arms A and B were overall well balanced. More patients in Arm B had surgery for recurrent disease, whereas more patients in Arm A had steroids at study entry. At the time of database closure (June 30, 2013), 87 deaths were documented, 84 were attributed to tumor progression, and 3 documented with unknown course. No patient was still on study treatment. Four patients had not reached the primary endpoint of TTF (Fig. 1).

Safety and tolerability
All adverse events were categorized by system organ class and graded according to CTCAE. There was no relevant difference between both arms regarding the frequency and severity of adverse events in the hematologic system. Profound lymphopenia was the most common hematologic toxicity, 19% in Arm A and 29% in Arm B [Supplementary Table S1]. Severe infections, however, were rare. Nonhematologic adverse events, for example, disorders of the gastrointestinal system, nervous system, metabolism, respiratory system, skin, cardiovascular system, or musculoskeletal system occurred at similar rates in both treatment arms and were overall infrequent.

Table 1. Patient characteristics before enrollment

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
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<tbody>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (y)</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td>Range (y)</td>
<td>21–62</td>
<td>37–59</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34 (65%)</td>
<td>35 (66%)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (35%)</td>
<td>18 (34%)</td>
</tr>
<tr>
<td>MGMT promoter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated</td>
<td>28 (53.8%)</td>
<td>31 (58.5%)</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>24 (46.2%)</td>
<td>22 (41.5%)</td>
</tr>
<tr>
<td>Surgery for recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>36 (69.2%)</td>
<td>41 (77.4%)</td>
</tr>
<tr>
<td>Yes</td>
<td>16 (30.8%)</td>
<td>12 (22.6%)</td>
</tr>
<tr>
<td>Surgery for recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (61.5%)</td>
<td>33 (62.3%)</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (38.5%)</td>
<td>20 (37.7%)</td>
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<tr>
<td>Survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median PFS (mo, 95% CI)</td>
<td>12.0 (8.8–17.0)</td>
<td>11.0 (9.2–12.9)</td>
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<tr>
<td>KPS at study entry</td>
<td></td>
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<tr>
<td>90–100</td>
<td>30 (57.7%)</td>
<td>30 (56.6%)</td>
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<tr>
<td>70–80</td>
<td>15 (28.8%)</td>
<td>16 (30.2%)</td>
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<tr>
<td>&lt;70</td>
<td>7 (13.5%)</td>
<td>7 (13.2%)</td>
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<td>Steroids at study entry</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>16 (30.8%)</td>
<td>12 (22.6%)</td>
</tr>
<tr>
<td>No</td>
<td>36 (69.2%)</td>
<td>41 (77.4%)</td>
</tr>
<tr>
<td>Number of maintenance TMZ cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No data</td>
<td>1 (1.9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>&lt;3</td>
<td>9 (17.3%)</td>
<td>12 (22.6%)</td>
</tr>
<tr>
<td>4–6</td>
<td>32 (61.5%)</td>
<td>33 (62.3%)</td>
</tr>
<tr>
<td>7 or more</td>
<td>10 (19.2%)</td>
<td>8 (15.1%)</td>
</tr>
<tr>
<td>Time since last TMZ administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 mo</td>
<td>20 (38.5%)</td>
<td>20 (37.7%)</td>
</tr>
<tr>
<td>≥2 mo</td>
<td>32 (61.5%)</td>
<td>33 (62.3%)</td>
</tr>
</tbody>
</table>

Further analyses and endpoints
Statistical analyses
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Outcome by treatment and MGMT status
All clinical outcome parameters were comparable in Arms A and B (Fig. 2; Table 2). Median TTF was below 2 months, whereas median OS from first intake of study drug was in the range of 10 months. The P value from the permutation test (P = 0.488) was close enough to the P value using partial likelihood from the Cox model (P = 0.485) to justify taking the latter one for all other analyses. There were two CR (4%) and two PR (4%) in Arm A and four CR (8%) and four PR (8%) in Arm B by local assessment (P = 0.68) in response to the study treatment. The median duration of

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the six CR was 4.5 months (95% CI, 1.8–11.0). The median duration of the six PR was 3 months (95% CI, 1.8–13.6). TTF was diagnosed because of PD in all but 3 patients, confirming that tolerability was good. One patient developed wound infection at day 26, necessitating temozolomide discontinuation, and 2 patients died without documented PD. Age was not prognostic.

As required per protocol, MGMT status from primary or recurrent tumor was available for all patients. MGMT promoter methylation was strongly associated with superior TTF and all other outcome parameters (Fig. 2; Table 3). The TTF difference between patients with versus without MGMT promoter methylation was more prominent in Arm B than in Arm A (Supplementary Table S2). Overall survival from initial histologic diagnosis of glioblastoma was 25.4 months (95% CI, 17.8–32.3) in Arm A and 22.7 months (95% CI, 18.5–27.2) in Arm B. This shows that patients enrolled into randomized trials for recurrent glioblastoma represent a selected population.

Central radiology review
Serial MRI of 85 patients was available for post hoc central review of progression. All these patients had measurable disease at baseline. The time point of progression was centrally confirmed in 81 patients. It was antedated 1 scan in 2 patients and not confirmed in 2 patients; 0 of 1 CR and 2 of 3 PR were confirmed. Insufficient scans were provided for the other 12 patients considered objective responders locally.

Outcome by pre-exposure to TMZ
We also separated the patient populations by intensity and interval of pre-exposure to TMZ. Administration of more than six cycles of maintenance temozolomide is uncommon in Europe (Table 1). To this end, we compared patients with intervals below \( n = 40 \) or above 2 months \( n = 65 \) since their last temozolomide intake as specified in the study protocol. Four of six CR and all six PR were noted in the latter group. Furthermore, there was significantly improved outcome in patients with a longer delay since the last administration of TMZ, more prominent in Arm A than in Arm B, and largely confined to patients with MGMT promoter methylation (Supplementary Table S3).

MMSE and quality of life
Serial assessments of MMSE and quality of life using EORTC QLQ-C30 and QLQ-BN20 were grouped into (i) pretreatment, (ii) during study treatment, and (iii) after study treatment assessments. For the latter two time intervals carrying multiple measures, we determined patient-wise minimum, median, and maximum scores. The MMSE as a surrogate measure of cognitive function...
remained stable during treatment and did not exhibit a decline after the end of study treatment as long as data were captured (Supplementary Fig. S1 and Supplementary Table S4). There was relatively little difference in quality of life assessed by QLQ-C30 and QLQ-BN20 when compared after the first 90 days of study treatment (Supplementary Table S5). Treatment-by-time interaction indicated that quality of life developments were somewhat more favorable in Arm B, with significant differences for pain (Supplementary Table S6). Although most scales are deteriorating over time (positive slope terms in either arm), the lack of major decline over time may result from the low number of assessments after the end of study treatment (Supplementary Table S7).

Multivariate modeling of outcome

Cox proportional hazards modeling for TTF revealed MGMT promoter methylation status and time interval from last temozolomide exposure as independent prognostic factors, whereas no such role was identified for age, KPS, surgery for recurrent tumor

Table 2. Outcome by treatment arm

<table>
<thead>
<tr>
<th></th>
<th>Arm A (7/14)</th>
<th>Arm B (21/28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td>Events</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Median TTF</td>
<td>1.8 (1.8–3.2)</td>
<td>1.95 (1.84–3.44)</td>
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<tr>
<td>Median survival from first study drug administration</td>
<td>9.8 (6.6–13.0)</td>
<td>10.6 (8.1–11.7)</td>
</tr>
<tr>
<td>TTF-6</td>
<td>17.1 (8.2–28.8)</td>
<td>25.0 (14.3–37.3)</td>
</tr>
<tr>
<td>PFS-6</td>
<td>17.1 (8.2–28.8)</td>
<td>25.0 (14.3–37.3)</td>
</tr>
<tr>
<td>Survival rate at 12 months from first study drug administration</td>
<td>41.0 (26.7–54.8)</td>
<td>32.7 (20.2–45.9)</td>
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</table>
before enrolment (Table 4). Steroid administration at study entry, body surface area, body weight, red or white blood cell or lymphocyte counts, hemoglobin or hematocrit at study entry were not prognostic for TTF (data not shown). Similar results were obtained when Cox proportional hazards modeling was applied to PFS, whereas only MGMT status was prognostic for survival from first study drug administration (data not shown).

Discussion

Standards of care in recurrent glioblastoma are not well defined. This definitive report of the phase II randomized DIRECTOR trial indicates that temozolomide rechallenge is a valid treatment option for patients with recurrent glioblastoma with, but not without, MGMT promoter methylation.

The optimal dosing of temozolomide in glioblastoma became a dominant topic in the first decade of this century in Neuro-Oncology, in part reflecting the lack of promising alternative drugs, in part also reflecting the consideration that temozolomide activity is critically limited by chemoresistance afforded by MGMT (27, 28). MGMT promoter methylation is observed in 30% to 40% of glioblastomas, presumably resulting in decreased MGMT gene expression in the MGMT-promoter-methylated tumor cells, thereby rendering glioblastomas more sensitive to TMZ. However, a predictive role of MGMT promoter methylation for benefit from alkylating chemotherapy including temozolomide has only been defined for glioblastoma, whereas MGMT promoter methylation is prognostic for better outcome with either radiotherapy or chemotherapy in patients with anaplastic gliomas (29, 30). This difference in biologic significance of MGMT promoter methylation is probably not related to grade of malignancy per se, but to the differential distribution of isocitrate dehydrogenase (IDH) mutations among these tumors. MGMT promoter methylation associated with IDH mutation and the glioma-associated CpG island methylation phenotype (G-CIMP) does not have the same significance as MGMT promoter methylation on the wild-type IDH background of glioblastoma (31, 32).

Because temozolomide depletes MGMT protein in peripheral blood mononuclear cells (33) and presumably glioblastoma cells, too, it was tempting to speculate that higher doses of temozolomide given over a longer time frame would eventually deplete MGMT. Accordingly, it was assumed that specifically patients with glioblastomas lacking MGMT promoter methylation might benefit from dose-intense temozolomide regimens. In addition and in parallel to DIRECTOR, two further trials explored the potential role of temozolomide dose intensification in glioblastoma. For the newly diagnosed setting, the hypothesis that more temozolomide might deplete MGMT and confer a survival benefit was falsified by the RTOG 0525 trial which confirmed the strong prognostic role of the MGMT status in TMZ-treated patients, but showed no difference between standard-dosed temozolomide or a 3 weeks on/1 week off regimen for six to 12 cycles in the maintenance phase, also not when the analysis was stratified for MGMT status (34). The BR12 trial analyzed the same two regimens in comparison with procarbacine, CCNU, and vincristine (PCV) in recurrent malignant glioma and similarly observed no difference between the three arms (35). However, this trial had enrolled chemonaive patients with WHO grade III or IV gliomas, which does not inform about the current situation in clinical practice where recurrent or progressive glioblastoma patients have commonly been pre-treated with TMZ/RT—TMZ.

The DIRECTOR trial reports a median TTF in the range of 2 months and yields overall no evidence that there are clinically relevant differences between the two dosing regimens, about either efficacy, safety, or tolerability (Fig. 2; Tables 2 and 3). Importantly, the dosing regimens were both confirmed to be feasible, given that PD was driving TTF in all, but one patient (s). The PFS-6 rate of 21% is in the range of previously reported figures of 11% to 24% (11, 12, 36). In contrast with the RESCUE trial (11), we observed a better PFS in patients off temozolomide for 2 months or more (Supplementary Table S3). Of note, it is uncommon in Europe to give temozolomide for more than 6 months (Table 1). These considerations indicate that some of the patients escalated to dose-intensified temozolomide regimens early in the disease course in RESCUE as well as in our previous reports (37) were in fact suffering from pseudoprogression, artificially raising the PFS-6 rate. Increased awareness of pseudoprogression may thus explain an apparent decrease in PFS-6 rates with temozolomide rechallenge in contemporary studies (12), and challenges all cross trial comparisons to older series. Moreover, differences in the PFS-6 figures for temozolomide rechallenge—and probably CCNU, too are likely to be related to the proportion of patients with tumors with MGMT promoter methylation in these studies, for example, PFS-6 was 26% with versus 0% without MGMT promoter methylation in the control arm of

Table 3. Outcome by MGMT promoter methylation status

<table>
<thead>
<tr>
<th></th>
<th>MGMT-unmethylated glioblastoma</th>
<th>MGMT-methylated glioblastoma</th>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Events</td>
</tr>
<tr>
<td>Median TTF</td>
<td>59</td>
<td>56</td>
</tr>
<tr>
<td>Median survival from first study drug administration</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>PFS-6</td>
<td>58</td>
<td>54</td>
</tr>
<tr>
<td>Survival rate at 12 months from first study drug administration</td>
<td>53</td>
<td>41</td>
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</table>

Table 4. Multivariate analyses of predictors for inferior TTF

<table>
<thead>
<tr>
<th></th>
<th>HR and 95% CI</th>
<th>P</th>
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<tr>
<td>Arm A vs. arm B</td>
<td>1.16 (0.76–1.76)</td>
<td>0.485</td>
</tr>
<tr>
<td>Age at study entry 50+ vs. 18–49 y</td>
<td>1.27 (0.76–2.00)</td>
<td>0.391</td>
</tr>
<tr>
<td>Time interval since last TMZ: &lt; vs. ≥ 2 mo</td>
<td>1.60 (1.00–2.55)</td>
<td>0.036</td>
</tr>
<tr>
<td>Salvege surgery: no vs. yes</td>
<td>1.02 (0.65–1.57)</td>
<td>0.945</td>
</tr>
<tr>
<td>KPS 50–60 vs. 90–100</td>
<td>1.03 (0.52–1.92)</td>
<td>0.786</td>
</tr>
<tr>
<td>KPS 70–80 vs. 90–100</td>
<td>1.05 (0.63–1.71)</td>
<td>0.841</td>
</tr>
<tr>
<td>MGMT promoter: unmethylated vs. methylated</td>
<td>1.76 (1.11–2.82)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

NOTE: HRs as exponential function of parameter estimates and CI. Estimates from a Cox model containing arm, treatment, age, time since last TMZ, salvage surgery: no vs. yes, KPS, and MGMT promoter methylation status as explanatory variables. Other factors, which are mentioned in the text, were included as additional variables in turn (one at a time). P values depicted in bold indicate a statistical significance level of <0.05.
the BELOB trial (38). The major limitations of the DIRECTOR trial are the relatively small sample size and the premature trial closure, which allows for less definitive conclusions. Yet, despite the lower than planned sample size and the premature trial closure, the likelihood of a major difference in efficacy between the different temozolomide schedules is very low.

In fact, the most important result of DIRECTOR is the strong prognostic role of the MGMT promoter methylation status in patients rechallenged with temozolomide that has not previously been studied prospectively in an adequately sized patient population. In contrast, age and KPS were not prognostic, likely reflecting preselection of patients enrolled into randomized trials for recurrent as opposed to newly diagnosed glioblastoma enriching patients with a similar, relatively favorable outcome. MGMT status was centrally assessed and was available for all patients. Although there was only a moderate advantage in median TTF of 3.2 versus 1.8 months in patients with MGMT promoter-methylated versus unmethylated tumors, PFS-6 was increased 5.8-fold, and OS at 12 months 2.4-fold (Fig. 2; Table 3). Yet, given the absence of an inactive comparator or a placebo, it cannot be excluded that MGMT promoter methylation is merely prognostic. Thus, bevacizumab alone was associated with superior PFS at 6 months in patients with tumors versus without MGMT promoter methylation in the BELOB trial, too (38), supporting a prognostic role of MGMT promoter methylation in recurrent glioblastoma. Randomization between temozolomide and placebo and the demonstration of benefit from temozolomide exclusively in patients with tumors with MGMT promoter methylation would be required for definitive confirmation, but is neither feasible nor ethical in patients with recurrent glioblastoma.

The findings of the DIRECTOR trial have implications for current clinical practice. On the basis of DIRECTOR, temozolomide rechallenge should no longer be considered for patients with tumors lacking MGMT promoter methylation, but remains a viable option for patients with MGMT promoter-methylated glioblastomas, notably after a drug-free interval of 2 months or more. Whether temozolomide given at 5 out of 28 days would be as effective as dose-intense regimens in patients recurring after a drug-free interval, remains uncertain, but the 5 of 28 regimen may be preferred in that setting because of better tolerability. More importantly, it may be speculated that a similarly profound prognostic effect of the MGMT status would have been seen, had the patients been treated with nitrosoureas instead of temozolomide (38). If confirmed, this would call for MGMT testing of primary or recurrent tumor and stratification for all, notably smaller randomized recurrent glioblastoma trials carrying an alkylator control arm because imbalances in the distribution of patients with MGMT-unmethylated versus MGMT-methylated tumors could severely bias outcome. In conclusion, DIRECTOR supports stratified treatment algorithms based on MGMT promoter methylation status in recurrent glioblastoma and advocates an alkylator regimen, including dose-dense temozolomide, as the most appropriate option for patients with glioblastoma harboring MGMT promoter methylation.

Disclosure of Potential Conflicts of Interest

M. Weller reports receiving commercial research grants from and is a consultant/advisory board member for MSD. G. Tabatabai reports receiving travel grant from MSD. J.P. Steinbach reports receiving speakers bureau honoraria from Medac, and is a consultant/advisory board member for Mundipharma and Roche. U. Herrlinger reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Merck Serono and Roche. U. Schlegel reports receiving speakers bureau honoraria from GlasoSmithKline and Medac, and is a consultant/advisory board member for Roche. R. Stupp is a consultant/advisory board member for Merck, Novartis, and Roche. J. Huisng reports receiving commercial research support from Onyx GmbH & Co. KG. G. Reifenberger reports receiving commercial research grants Roche, and reports receiving speakers bureau honoraria from Amgen and Roche. W. Wick reports receiving speakers bureau honoraria from MSD, Prime Oncology and Roche, and is a consultant/advisory board member for Apogenics and Roche. No potential conflicts of interest were disclosed by the other authors.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Weller, B. Kastner, J. Felsberg, A. Wick, O. Schnell, P. Hau, H.-G. Wirsching, O. Bähr, C. Marosi, J. Hüsing

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Other (study coordination): G. Tabatabai

Other (contribution to translational projects): P. Hau

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