Clinical Cancer Research

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/m^2 per day)/one week off] or Arm B [3 weeks on (80 mg/m^2 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. O^6-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.*

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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).

Standards of care at progression are less well defined (6): Depending on approval status, individual patient and tumor factors, and local preference, the most commonly used systemic therapeutic approaches include nitrosoureas such as lomustine (CCNU) which has become the standard of care in randomized clinical trials for recurrent glioblastoma (7, 8), temozolomide rechallenge using various regimens (9–12), and bevacizumab (13, 14). The need for prospective assessment of 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 (**D**ose-**I**ntensified **Rec**hallenge with **T**emozolomide, **O**ne Week on One Week Off versus Three Weeks on One Week Off in Patients with Progressive or **R**ecurrent Glioblastoma) trial, which sought to explore the activity of two widely used regimens of dose-intense temozolomide for recurrent glioblastoma, 1 week on/1 week off (7/14; ref. 9) versus 3 weeks on/1 week off (10).

Patients and Methods

Study design

DIRECTOR (NCT 00941460) was designed as a prospective, open-label, randomized, two-arm trial of two competing temozolomide dosing regimens for patients with glioblastoma at first relapse or progression. The primary objective was to show the superiority of Arm A (1 week on/1 week off) over Arm B (3 weeks on/1 week off), the primary endpoint being time-to-treatment failure (TTF). Major inclusion criteria were: progressive or recurrent glioblastoma documented by MRI no earlier than 180 days after first surgery for glioblastoma and no earlier than 90 days after completion of radiotherapy (patients with progression outside the radiation field were also not allowed to be entered into the trial unless these time frames were respected); histologic diagnosis of glioblastoma; tissue available for the determination of O^6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation in the primary or in the recurrent tumor; prior treatment with TMZ/RT, and at least two cycles of maintenance temozolomide (5/28); informed consent; age 18 to 80 years; Karnofsky performance score $\geq$ 50%; absolute neutrophil counts > 1,500/μL; platelet counts > 100,000/μL; hemoglobin > 10 g/dL; serum creatinin < 1.5-fold upper normal range; ASAT or ALAT < 3-fold upper normal range unless attributed to anticonvulsants; alkaline phosphatase < 3-fold upper normal range; women with childbearing potential must have a negative serum pregnancy test $\leq$14 days before study enrollment. Obligatory *MGMT* testing of the recurrent tumor as opposed to the primary tumor tissue was initially required, but no longer requested when it became clear that the result of the *MGMT* status determination rarely changes in the course of disease (17). All patients gave written informed consent, and the study was approved by the local ethical committees and competent authorities. The trial was prematurely closed for withdrawal of support after the merger of Schering Plough with Merck, Sharp & Dohme. Databank closure was on June 30, 2013.

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 vital glioblastoma tissue with an estimated tumor cell content $\geq$80%. The *MGMT* promoter methylation status was determined by methylation-specific PCR (MSP) and evaluated as reported (19). This MSP assays had been used in several previous studies (20–22) and was proven to show high concordance with results obtained by the MSP assay of MDxHealth S.A. (22) and DNA pyrosequencing (17).

Study treatment

Patients were allocated either to the 1 week on/1 week off regimen (7/14, Arm A) or to the 3 weeks on/1 week off regimen (21/28, Arm B) of dose-intensified temozolomide using a treatment allocation algorithm (23) with a probability of a minimizing allocation set at 0.9. Arm A patients were treated at an initial dose of 120 mg/m^2 unless there had been grade III or IV myelotoxicity with conventional temozolomide (5/28) previously. These patients were started at 90 mg/m^2. Temozolomide was given orally on days 1 to 7 and 15 to 21. Arm B patients started with an initial dose of 80 mg/m^2 unless there had been significant myelotoxicity with conventional temozolomide (5/28) previously. These patients started at 60 mg/m^2. Temozolomide was given orally on days 1 to 21. A treatment cycle was defined as 2 completed weeks of temozolomide within 4 weeks in Arm A and as 3 weeks of continuous temozolomide within 4 weeks in Arm B. Dose modifications were foreseen according to hematologic parameters as outlined in Supplementary Table S1.

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 in the protocol (24). The primary endpoint, TTF, was calculated from randomization to any of the following: progressive disease defined by Macdonald criteria (24), death for any reason, or toxicity leading to discontinuation of study treatment for any reason. Secondary endpoints included progression-free survival (PFS) calculated as the time from randomization to the first documented evidence of progression of disease, survival from randomization, and efficacy parameters in subgroups defined by *MGMT* status. Radiologic progression was evaluated at each center and also centrally verified *post hoc* (blindly to previous results).

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 performance score (KPS) 50–60, 70–80, or 90–100] along with the variable for treatment. Because *P* values from permutation tests were sufficiently close to those based on partial likelihoods as routinely used in parameter estimation in the Cox model, the latter were used for all subgroup analyses. Secondary analyses with respect to time-to-event variables were done using Cox proportional hazards models when considering multiple explanatory variables. Log-rank or bootstrap tests to compare median times and parametric tests with standard error approximation were used for univariate tests, especially when comparing rates at fixed time points. With respect to categorical variables, we used the Fisher exact test or logistic regression, and with respect to continuous variables linear regression models. Specifically, quality of life was analyzed using a linear mixed regression model with patient as random effect. All *P* values, statistical tests, and confidence intervals (CI) beyond the analysis of the primary criterion were not corrected for multiplicity and are to be interpreted as exploratory.

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 databank 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).

Table 1. Patient characteristics before enrollment

	Arm A 7/7 *N* = 52	Arm B 21/7 *N* = 53
Age at diagnosis		
Median (y)	58	56
Range (y)	21–62	37–59
Gender		
Male	34 (65%)	35 (66%)
Female	18 (35%)	18 (34%)
MGMT promoter		
Methylated	28 (53.8%)	31 (58.5%)
Unmethylated	24 (46.2%)	22 (41.5%)
First-line therapy		
TMZ/RT	52 (100.0%)	53 (100.0%)
Number of maintenance TMZ cycles		
No data	1 (1.9%)	0 (0%)
≤3	9 (17.3%)	12 (22.6%)
4–6	32 (61.5%)	33 (62.3%)
7 or more	10 (19.2%)	8 (15.1%)
Time since last TMZ administration		
<2 mo	20 (38.5%)	20 (37.7%)
≥2 mo	32 (61.5%)	33 (62.3%)
Survival		
Median PFS (mo, 95% CI)	12.0 (8.8–17.0)	11.0 (9.2–12.9)
KPS at study entry		
90–100	30 (57.7%)	30 (56.6%)
70–80	15 (28.8%)	16 (30.2%)
<70	7 (13.5%)	7 (13.2%)
Steroids at study entry		
Yes	16 (30.8%)	12 (22.6%)
No	36 (69.2%)	41 (77.4%)
Surgery for recurrence		
Yes	29 (55.8%)	32 (60.4%)
No	23 (44.2%)	21 (39.6%)

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. Nonhematalogic 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.

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

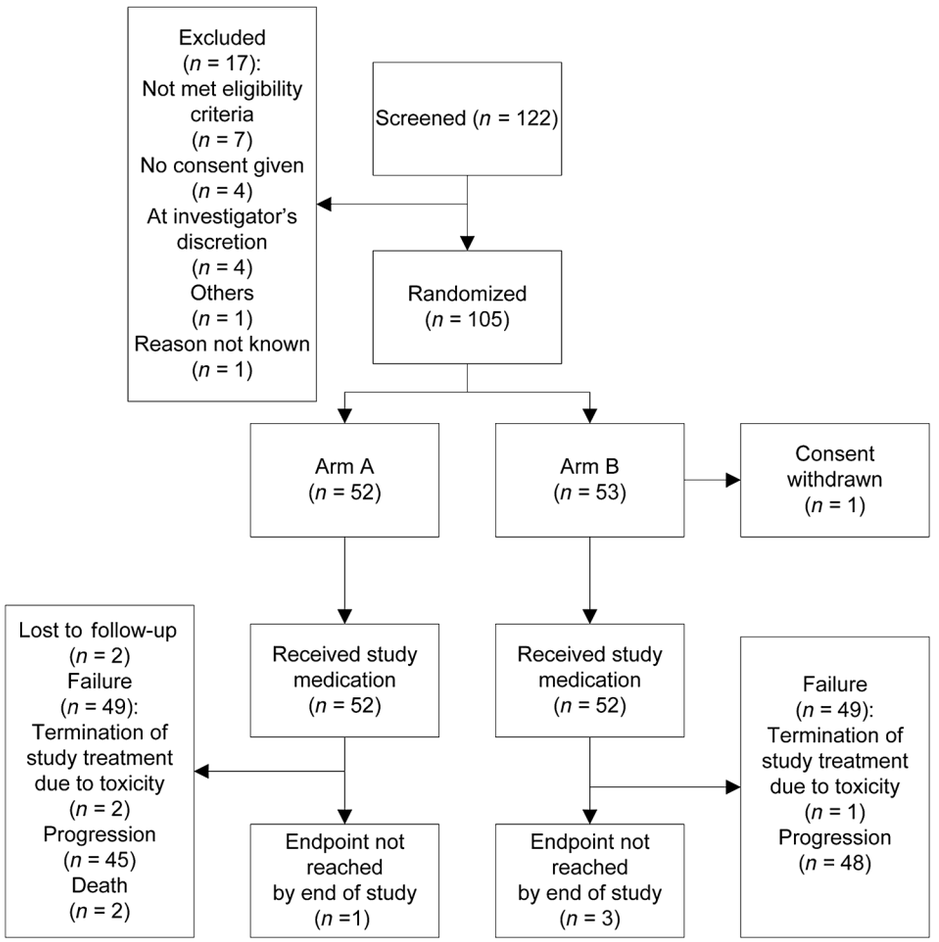

Figure 1.
CONSORT chart.

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

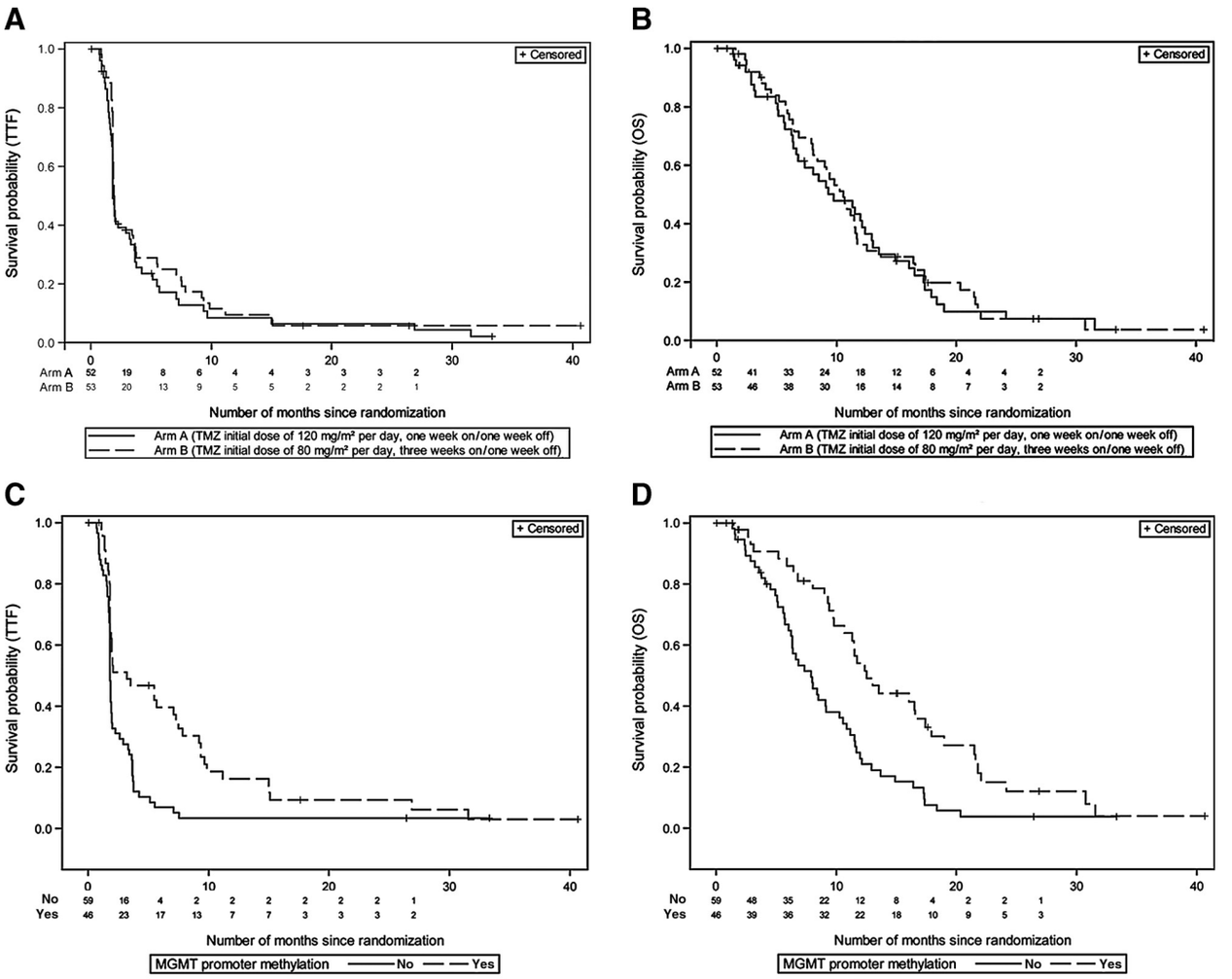

Figure 2.
Clinical outcome. TTF (A) and OS (B) in Arm A (7/14) versus Arm B (21/28). TTF (C) and OS (D) in patients without versus with *MGMT* promoter methylation.

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

	Arm A (7/14)			Arm B (21/28)			
	Patients	**Events**	**Time in mo (95% CI)**	**Patients**	**Events**	**Time in months (95% CI)**	***P***
Median TTF	52	49	1.8 (1.8–3.2)	53	49	1.95 (1.84–3.44)	0.37
Median survival from first study drug administration	52	42	9.8 (6.6–13.0)	53	45	10.6 (8.1–11.7)	0.78
			Rate in % (95% CI)			Rate in % (95% CI)	
TTF-6	50	42	17.1 (8.2–28.8)	52	39	25.0 (14.3–37.3)	0.33
PFS-6	50	42	17.1 (8.2–28.8)	52	39	25.0 (14.3–37.3)	0.33
Survival rate at 12 months from first study drug administration	45	27	41.0 (26.7–54.8)	49	33	32.7 (20.2–45.9)	0.40

Table 3. Outcome by *MGMT* promoter methylation status

	MGMT-unmethylated glioblastoma			*MGMT*-methylated glioblastoma			
	Patients	**Events**	**Time in months (95% CI)**	**Patients**	**Events**	**Time in months (95% CI)**	***P***
Median TTF	59	56	1.8 (1.8–2.0)	46	42	3.2 (1.8–7.3)	0.0014
Median survival from first study drug administration	59	50	7.9 (6.3–10.3)	46	36	12.5 (9.8–17.4)	0.0009
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	58	54	6.9 (2.2–15.3)	44	27	39.7 (25.5–53.5)	<0.0001
Survival rate at 12 months from first study drug administration	53	41	22.9 (12.7–34.9)	41	19	54.1 (37.8–67.8)	0.0013

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 methylator 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 pretreated 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 4. Multivariate analyses of predictors for inferior TTF

	HR and 95% CI	***P***
Arm A vs. arm B	1.16 (0.76–1.76)	0.485
Age at study entry 50+ vs. 18–49 y	1.27 (0.76–2.20)	0.381
Time interval since last TMZ: < vs. ≥ 2 mo	1.60 (1.00–2.55)	**0.036**
Salvage surgery: no vs. yes	1.02 (0.65–1.57)	0.945
KPS 50–60 vs. 90–100	1.03 (0.52–1.92)	0.786
KPS 70–80 vs. 90–100	1.05 (0.63–1.73)	0.841
MGMT promoter: unmethylated vs. methylated	1.76 (1.11–2.82)	**0.017**

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 closure of the study, which allows for less definitive conclusions. Yet, despite the lower than planned sample size and the premature trial closure, the likelyhood 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 with 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 a 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 Medac. O. Bahr is a consultant/advisory board member for Roche. J-T. Tonn 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 GlaxoSmithKline and Medac, and is a consultant/advisory board member for Roche. R. Stupp is a consultant/advisory board member for MSD/Merck, Novartis, and Roche. J. Husing reports receiving commercial research support from Oryx 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 Apogenix and Roche. No potential conflicts of interest were disclosed by the other authors.

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