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Letter to the Editor

MGMT promoter methylation determined by the MGMT-STP27 algorithm is not predictive for outcome to temozolomide in IDH-mutant anaplastic astrocytomas

O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation is an important predictor of response to alkylating chemotherapy in glioblastomas.[1] A common method to determine *MGMT* promoter status is with the MGMT-STP27 algorithm which is calculated from the methylation levels of two specific CpGs (cg12434587 and cg12981137) on Illumina DNA methylation arrays.[2] This algorithm was constructed with data from predominantly isocitrate dehydrogenase 1 and 2 (IDH)-wildtype glioblastomas but is often extrapolated to IDH-mutant astrocytomas. However, IDH-wildtype glioblastomas usually exhibit loss of heterozygosity (LOH) of chromosome 10, whereas this copy number change is uncommon in IDH-mutant astrocytomas.[3] This LOH is relevant because the *MGMT* gene is situated on chromosomal band 10q26, meaning that only one intact copy is left in most IDH-wildtype glioblastoma while two copies are present in IDH-mutant astrocytomas. Complete silencing of *MGMT* is most likely a prerequisite for efficacy of temozolomide treatment in high-grade glioma, since a reduced DNA repair (from O^6-methylguanine to guanine) makes tumor cells more susceptible to treatment with alkylating agents that induce these defaults (from guanine to O^6-methylguanine).[4,5] The presence of two intact alleles in IDH-mutant astrocytomas, therefore, may indicate that *MGMT* gene methylation and subsequent temozolomide effectiveness might differ from IDH-wildtype glioblastomas. The correlation of *MGMT* expression with the MGMT-STP27 algorithm in tumors likely to be IDH-mutant has been assessed before, but without correlation with clinical outcome.[2]

In a second interim analysis of the CATNON trial, efficacy was shown of adjuvant, but not concurrent, temozolomide in patients with IDH-mutant anaplastic astrocytoma.[6] We investigated whether the efficacy of adjuvant temozolomide in the CATNON study was correlated to *MGMT* promoter methylation as determined by the MGMT-STP27 algorithm. We identified 440 IDH-mutant anaplastic astrocytomas with available MGMT-STP27 data. Of these, 365 tumors (83.0%) were *MGMT*-methylated, 224 (50.9%) were treated with adjuvant temozolomide, and no differences were found in *MGMT* methylation per treatment group (χ^2 test: $P = .50$). The effect of adjuvant temozolomide on overall survival was similar between patients with *MGMT*-methylated and *MGMT*-unmethylated tumors (Figure 1). In a Cox proportional hazards model the interaction term of adjuvant temozolomide and *MGMT* promoter methylation was not significant ($P = .92$). Similar lack of predictive effect was observed when comparing patients treated with or without concurrent temozolomide (Figure 1B), or when comparing the radiotherapy alone arm to the other three study arms individually (Figure 1B), or even when comparing progression-free survival for the radiotherapy alone arm to the combination of the other three arms ($P = .11$). This illustrates that regardless of the timing of the temozolomide treatment, no correlation can be found between the MGMT-STP27 algorithm and temozolomide efficacy. All aforementioned analyses were performed with the standard cutoff (0.3582) for the MGMT-STP27 algorithm as derived from IDH-wildtype glioblastoma data.[2] We performed exploratory analysis of other cutoff values to correct for possible differences between IDH-mutant astrocytomas and IDH-wildtype glioblastomas. The MGMT-STP27 values of the CATNON samples displayed an expected bimodal distribution with the first and the second peak representing the unmethylated samples and methylated samples, respectively. Based on the lowest point between the peaks of the bimodal distribution, we estimated the optimal cutoff for these IDH-mutant astrocytomas to be 0.3349. This new cutoff was similar to the standard cutoff, and changing the cutoff value did not alter our conclusions.

Therefore, data on the CATNON trial samples indicate that there is no predictive value of the MGMT-STP27 algorithm in relation to treatment with temozolomide in IDH-mutant anaplastic astrocytomas. However, the current number of events is limited and more follow-up is needed. It remains to be determined if other CpGs on the *MGMT* promoter hold any predictive power, and whether testing for *MGMT* promoter status is clinically useful in IDH-mutant tumors.

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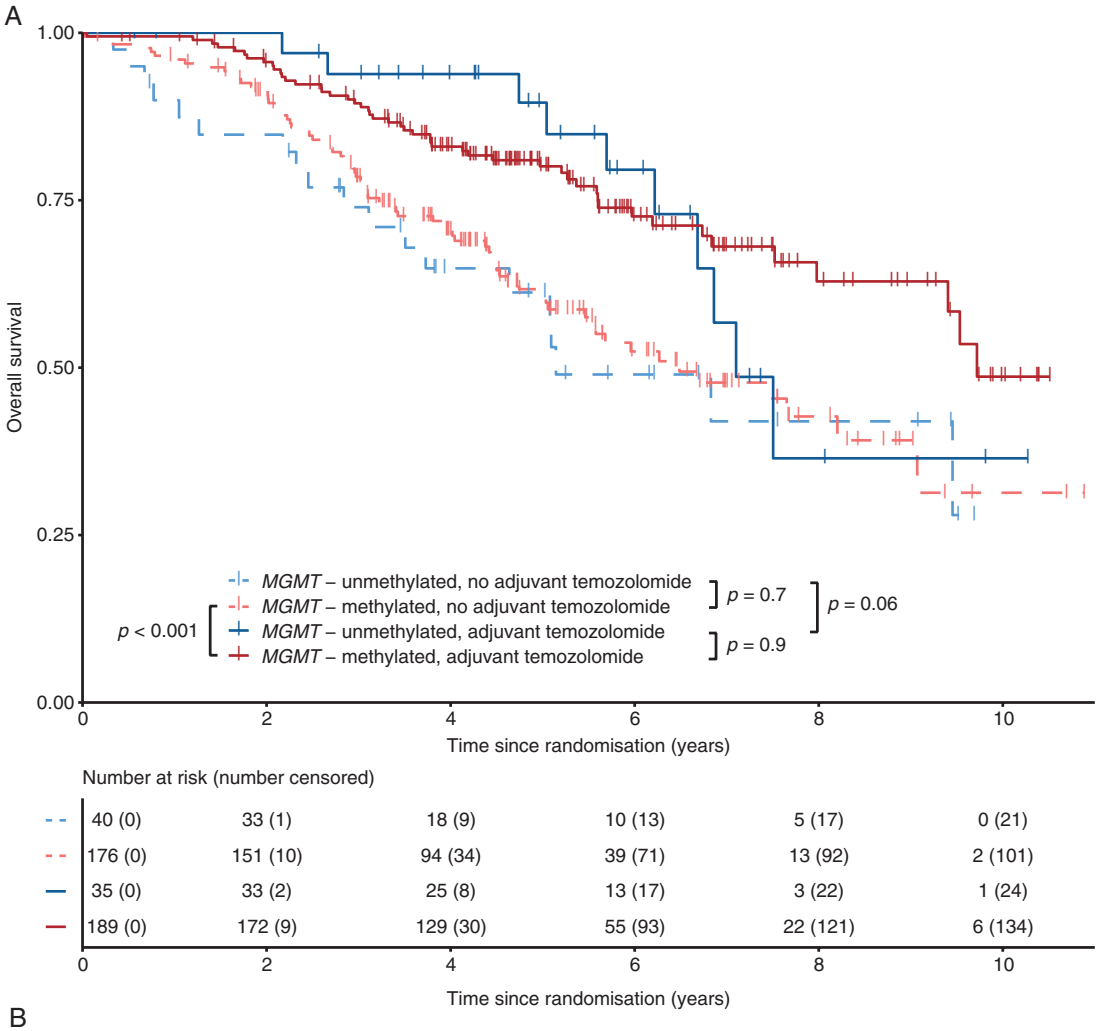

B

Treatment	*MGMT* status	*n*	Events	HR (95% CI)	Wald test
Concurrent temozolomide vs no concurrent temozolomide	Methylated	186 vs 179	53 vs 69	0.78 (0.55–1.12)	$p = 0.94$
	Unmethylated	38 vs 37	14 vs 15	0.74 (0.35–1.53)	
Adjuvant temozolomide vs no adjuvant temozolomide	Methylated	189 vs 176	49 vs 73	0.50 (0.34–0.72)	$p = 0.92$
	Unmethylated	35 vs 40	10 vs 19	0.48 (0.22–1.04)	
Radiotherapy with concurrent temozolomide vs radiotherapy alone	Methylated	91 vs 85	32 vs 41	0.77 (0.48–1.22)	$p = 0.96$
	Unmethylated	20 vs 20	9 vs 10	0.83 (0.33–2.07)	
Radiotherapy with adjuvant temozolomide vs radiotherapy alone	Methylated	94 vs 85	28 vs 41	0.50 (0.31–0.80)	$p = 0.99$
	Unmethylated	17 vs 20	5 vs 10	0.49 (0.17–1.43)	
Radiotherapy with concurrent and adjuvant temozolomide vs radiotherapy alone	Methylated	95 vs 85	21 vs 41	0.38 (0.23–0.65)	$p = 0.94$
	Unmethylated	18 vs 20	5 vs 10	0.35 (0.12–1.05)	

Fig. 1 Overall survival of IDH-mutant anaplastic astrocytomas in relation to *MGMT* promoter methylation and treatment with temozolomide. (A) Kaplan-Meier curves comparing patients treated with adjuvant temozolomide (±concurrent temozolomide) to patients that were not treated with adjuvant temozolomide (±concurrent temozolomide). (B) Cox proportional hazards models for different treatment modalities with tests for interaction.

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Authorship statement. All authors were responsible for the study design, data collection, data analysis, data interpretation, and contributed to and approved the final version of the manuscript.

On behalf of the EORTC Brain Tumor Group and the CATNON investigators, C. Mircea S. Tesileanu, Thierry Gorlia, Vassilis Golfinopoulos, Pim J. French, and Martin J. van den Bent

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