Neuro-Oncology Practice
10(2), 126–131, 2023 | https://doi.org/10.1093/nop/npac070 | Advance Access date 10 September 2022

The predictive value of partial *MGMT* promoter methylation for IDH-wild-type glioblastoma patients

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Abstract

Background. Glioblastoma patients with hypermethylation of the O^6-methylguanine-methyltransferase (*MGMT*) gene promoter have significantly improved survival when treated with temozolomide compared to patients with unmethylation of the *MGMT* promoter. However, the prognostic and predictive significance of partial *MGMT* promoter methylation is unclear.

Methods. The National Cancer Database was queried for patients newly diagnosed in 2018 with histopathologically confirmed isocitrate dehydrogenase (IDH)-wildtype glioblastoma. The overall survival (OS) associated with *MGMT* promoter methylation status was assessed using multivariable Cox regression with Bonferroni correction for multiple testing ($P < .008$ was significant).

Results. Three thousand eight hundred twenty-five newly diagnosed IDH-wildtype glioblastoma patients were identified. The *MGMT* promoter was unmethylated in 58.7% ($n = 2245$), partially methylated in 4.8% ($n = 183$), hypermethylated in 3.5% ($n = 133$), and methylated not otherwise specified (NOS; likely consisting predominantly of hypermethylated cases) in 33.0% ($n = 1264$) of cases. Among patients that received first-line single-agent chemotherapy (ie likely temozolomide), compared to partial methylation (referent), *MGMT* promoter unmethylation was associated with worse OS (hazard ratio [HR] 1.94; 95% confidence interval [95 CI]: 1.54–2.44; $P < .001$) in multivariable Cox regression adjusted for major prognostic confounders. In contrast, a significant OS difference was not observed between partially methylated promoters and either hypermethylated (HR 1.02; 95 CI: 0.72–1.46; $P = .90$) or methylated NOS (HR 0.99; 95 CI: 0.78–1.26; $P = .93$) promoters. Among IDH-wildtype glioblastoma patients who did not receive first-line chemotherapy, *MGMT* promoter methylation status was not associated with significant differences in OS ($P = 0.39$–0.83).

Conclusions. Compared to *MGMT* promoter unmethylation, partial methylation was predictive of improved OS among IDH-wildtype glioblastoma patients treated with first-line single-agent chemotherapy—supporting the use of temozolomide therapy in these patients.

Keywords

epidemiology | glioblastoma | methylation | O^6-methylguanine-DNA methyltransferase (MGMT) promoter

Glioblastoma, WHO grade 4, is the most common malignant primary tumor of the central nervous system and is associated with an especially poor prognosis, with patients having a median survival of 14–19 months.[1,2] Several biomarkers have been identified, such as O^6-methylguanine-methyltransferase (*MGMT*) promoter hypermethylation, that are associated with improved clinical outcome.[3,4] *MGMT* promoter methylation status is not only prognostic, but also predictive of

glioblastoma response to treatment with the alkylating chemotherapeutic agent temozolomide.[5]

Temozolomide causes cytotoxicity by transferring methyl groups to purine DNA bases, including the O^6 position of guanine, that results in DNA base mismatch, activation of the DNA mismatch repair pathway, persistent DNA double stranded breaks, and induction of apoptosis.[6] The *MGMT* gene encodes an enzyme that repairs DNA damage by removing methyl groups from O^6-methylguanine to its own cysteine residues. However, *MGMT* can be epigenetically silenced through methylation of CpG islands within the gene's promotor region, increasing susceptibility to alkylating DNA damage. Approximately 40% of isocitrate dehydrogenase (IDH)-wildtype glioblastomas are *MGMT* promoter (hyper)methylated.[1] Glioblastoma and gliosarcoma patients with (hyper)methylated *MGMT* promoters derive strong clinical benefit from treatment with alkylating chemotherapeutic agents (eg temozolomide or—commonly in the recurrent setting—lomustine).[3,5,7–9] By contrast, glioblastoma patients with unmethylated *MGMT* promoters experience limited survival benefit from temozolomide, and treatment with this chemotherapy may expose certain patient populations such as the elderly or frail to unnecessary toxicity. Together, these data highlight the importance of accurately characterizing *MGMT* promoter methylation status for predicting prognosis and informing the therapeutic approach.[4,10]

The methodology and cutoff values used to characterize *MGMT* promoter methylation status vary across laboratories.[11] Although the *MGMT* promoter is frequently reported as either (hyper)methylated or unmethylated, an emerging body of literature has reported that the level of *MGMT* promoter methylation in a ~10% subset of glioblastomas falls within a "grey zone," alternatively described as partially methylated, weakly methylated, inconsistently methylated, or having low, faint, or intermediate methylation.[12–19] The precise terminology for these "grey zone" levels can vary depending on the assay used to determine the *MGMT* promoter methylation status. For simplicity, herein we will refer to these levels as partially methylated. The clinical significance and the utility of temozolomide treatment in glioblastomas with partial *MGMT* promoter methylation are unclear. Starting for brain tumors patients diagnosed in 2018, the US cancer registries implemented a new "O^6-Methylguanine-Methyltransferase (*MGMT*)" promoter methylation site-specific data item which, for the first time, reported partial/low/hypo methylation.[1] To address limitations in our understanding of the prognostic and predictive significance of partial *MGMT* promoter methylation in newly diagnosed IDH-wildtype glioblastomas, we compared their outcome data to *MGMT* promoter (hyper)methylation and unmethylation using the US National Cancer Database (NCDB)—stratified by treatment with or without first-line chemotherapy.

Methods

The NCDB reports data for more than 85% of patients with newly diagnosed primary malignant brain tumors in the United States. For brain tumor patients diagnosed starting in 2018, data were reported for a new "O^6-Methylguanine-Methyltransferase (*MGMT*)" promoter methylation site-specific data item, coded as either: 0) "unmethylated *MGMT*" or "*MGMT* methylation absent/not present" (herein referred to as unmethylated); 1) "Partial methylated", "Hypomethylated", or "*MGMT* methylation present, low level" (herein referred to as partially methylated); 2) "Hypermethylated", or "*MGMT* methylation present, high level" (herein referred to as hypermethylated); or 3) "*MGMT* methylation present, level unspecified" (herein referred to as methylated, not otherwise specified [NOS]). We suspect that the latter category was comprised primarily of *MGMT* hypermethylated cases, as the *MGMT* promoter is often reported in a simplified, binary (hyper) methylated/unmethylated classification scheme. Cancer registrars were instructed to use the pathology report, or specialty or reference laboratory report as the source documentation for encoding *MGMT* promoter methylation status. The coding instructions additionally included a note that the physician statement of the *MGMT* methylation status could also be used to code this data item. The source of *MGMT* status documentation for each patient was not reported by the NCDB.

2018 was also the latest year with overall survival (OS) data reported by the NCDB. We therefore identified all patients from the NCDB that were newly diagnosed in 2018 with a histopathologically confirmed IDH-wildtype glioblastoma (ICD-O-3 9440/3 and Brain Molecular Markers code 05), WHO grade 4, of the brain (site C71.0–C71.9). Patients were excluded if they had a prior diagnosis of cancer, did not have surgery, or if they received all of their management at a different institution from the one that reported data to the NCDB. The NCDB does not report information about the method of *MGMT* promoter methylation detection nor the laboratory cutoff values used to distinguish unmethylated, partially methylated, and hypermethylated tumors.

OS was measured from the date of initial diagnosis to the date of death, or censored at last follow-up, and estimated using Kaplan-Meier techniques. Multivariable Cox regression was used to assess the association between *MGMT* promoter methylation status and OS. Variables with prognostic value were included to adjust for potential confounding, including age at diagnosis, sex, maximal dimension of the tumor, radiotherapy, and extent of resection (categorized as biopsy, subtotal resection [STR], or gross total resection [GTR]). OS was evaluated separately for patients receiving first-line single-agent chemotherapy (ie likely temozolomide) and those not receiving first-line chemotherapy (reported as a supplemental analysis). A two-sided study-wide α level of 0.05 was designated as significant, using a Bonferroni correction for multiple testing. Six hypotheses were prospectively designated, so each had a corrected *P* value threshold of .008 for significance. In the multivariable analysis, p values were only reported for the primary association of interest of *MGMT* promoter methylation status (partially methylated as the referent) with OS. Confidence intervals (CI) were provided for all other associations. All analyses were conducted using Stata (v17.0, StataCorp) This study was approved by the Mass General Brigham institutional review board (#2015P002352) and conducted in accordance with the

Declaration of Helsinki. The NCDB Participant User Files contain deidentified national data for which consenting was not applicable.

Results

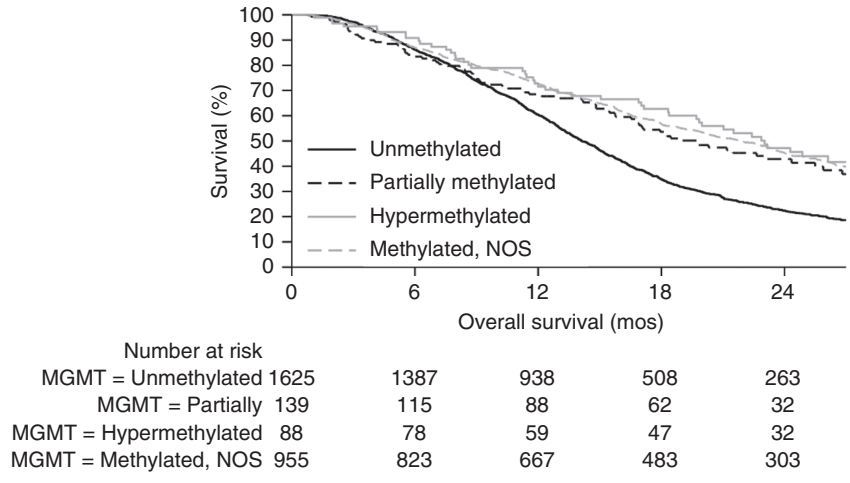
In 2018, there were 3825 patients reported in the NCDB with newly diagnosed, histopathologically confirmed IDH-wildtype glioblastoma, WHO grade 4, who had documented *MGMT* promoter methylation status and who met our inclusion and exclusion criteria. Among these patients, the *MGMT* promoter was unmethylated in 58.7% ($n = 2245$), partially methylated in 4.8% ($n = 183$), hypermethylated in 3.5% ($n = 133$), and methylated NOS in 33.0% ($n = 1264$) of cases. Of the 2807 patients who received single-agent chemotherapy (ie likely temozolomide), the *MGMT* promoter was unmethylated in 57.9% ($n = 1625$), partially methylated in 5.0% ($n = 139$), hypermethylated in 3.1% ($n = 88$), and methylated NOS in 34.0% ($n = 955$) of cases. Baseline patient and tumor characteristics by *MGMT* promoter status were reported in Supplementary Table 1.

The unadjusted Kaplan-Meier OS curves stratified by *MGMT* promoter methylation status among newly diagnosed, IDH-wildtype glioblastoma patients that received first-line single-agent chemotherapy (ie likely temozolomide) are shown in Figure 1. In multivariable Cox regression adjusted for major prognostic confounders, compared to partial methylation (referent), unmethylated *MGMT* promoters remained associated with worse OS (hazard ratio [HR] 1.94; 95% confidence interval [95 CI]: 1.54–2.44; $P < .001$) (Table 1). In contrast, a significant OS difference was not observed between partially methylated promoters and either hypermethylated (HR 1.02; 95 CI: 0.72–1.46; $P = .90$) or methylated NOS (HR 0.99; 95 CI: 0.78–1.26; $P = .93$) promoters.

Among IDH-wildtype glioblastoma patients who did not receive first-line chemotherapy, *MGMT* promoter methylation status was not associated with significant differences in OS ($P = .39$–$.83$; Supplementary Table 2).

Discussion

Although *MGMT* promoter hypermethylation has a well-established prognostic and predictive role in glioblastoma, the significance of partial *MGMT* promoter methylation is less clear. In a national analysis of newly diagnosed, IDH-wildtype glioblastoma patients who were treated with first-line single-agent chemotherapy (ie likely temozolomide), we provide evidence that partial methylation of the *MGMT* promoter was associated with improved OS as compared to their unmethylated counterparts—with an OS comparable to that of *MGMT* promoter hypermethylation. In addition, for patients not treated with chemotherapy in the first-line setting, a significant difference in OS by *MGMT* promoter status was not observed. Our results suggest that IDH-wildtype glioblastoma patients with at least partial *MGMT* promoter methylation may clinically benefit from treatment with temozolomide (ie partial *MGMT* promoter methylation is predictive of response to temozolomide) and that in the absence of first-line single-agent chemotherapy, partial *MGMT* promoter methylation was not prognostic. The latter finding stands in contrast with prior reports that found *MGMT* promoter hypermethylation to be prognostic independent of temozolomide treatment—suggesting that further research into the prognostic role of *MGMT* promoter methylation status is warranted.[5,20] Furthermore, the results of our study have implications for how laboratories should report *MGMT* promoter methylation values. Because laboratories and assays do not have standardized cutoff criteria for reporting *MGMT* promoter



Number at risk					
MGMT = Unmethylated	1625	1387	938	508	263
MGMT = Partially	139	115	88	62	32
MGMT = Hypermethylated	88	78	59	47	32
MGMT = Methylated, NOS	955	823	667	483	303

Fig. 1 Overall survival by *MGMT* promoter methylation status among newly diagnosed, IDH-wildtype glioblastoma patients who received first-line single-agent chemotherapy. Unadjusted Kaplan-Meier overall survival estimates by *MGMT* promoter methylation status with underlying number-at-risk table. Single-agent chemotherapy likely overwhelmingly represented temozolomide. NOS = not otherwise specified.

Table 1 Multivariable Cox Regression of Overall Survival Associated With *MGMT* Promoter Methylation Status Among Newly Diagnosed, IDH-Wild-Type Glioblastoma Patients Who Received First-Line Single-Agent Chemotherapy (ie Likely Temozolomide)

MGMT promoter status	HR	95% CI	*P*-value
Unmethylated	1.94	(1.54–2.44)	<.001
Partially methylated	Referent		
Hypermethylated	1.02	(0.72–1.46)	.90
Methylated, NOS	0.99	(0.78–1.26)	.93
Age at diagnosis, yrs			
<50	Referent		
50–59	1.33	(1.13–1.56)	
60–69	1.89	(1.63–2.19)	
≥70	2.99	(2.55–3.49)	
Sex			
Male	Referent		
Female	0.83	(0.76–0.91)	
Tumor size, cm			
<2	Referent		
≥2, <4	1.11	(0.91–1.35)	
≥4, <6	1.14	(0.94–1.39)	
≥6	1.29	(1.04–1.59)	
n/a	1.17	(0.95–1.45)	
Extent of resection			
Biopsy-only	Referent		
Subtotal resection	0.71	(0.63–0.80)	
Gross total resection	0.57	(0.50–0.63)	
First-line radiotherapy			
No	Referent		
Yes	0.49	(0.38–0.63)	

HR, hazard ratio; CI, confidence interval; NOS, not otherwise specified.

methylation status,[11] a subset of glioblastoma patients with partially methylated *MGMT* promoters may be dichotomized by some laboratories into either an unmethylated or (hyper)methylated status.[21] Revised *MGMT* promoter methylation reporting schemes should include these "grey zone" categories to better inform patient care and management.

There are several potential explanations for why glioblastoma patients with partially methylated *MGMT* promoters may benefit from treatment with temozolomide. Firstly, our findings may indicate that the laboratory cutoff values for characterizing *MGMT* promoter methylation status have not been optimized and standardized across the United States. Secondly, glioblastomas with hypermethylated *MGMT* promoters can be mischaracterized as partially methylated due to technical bias or tumor sampling error. Such cases might occur when tissue submitted for molecular testing has low tumor content, extensive necrosis, and/or dense infiltration by MGMT-expressing microglia and macrophages—among other sample quality and technical issues.[6,13,22,23] Thirdly, temozolomide may be clinically beneficial in glioblastomas with partial *MGMT* promoter methylation by inducing cytotoxicity in at least a subset of tumor cells and decreasing overall tumor burden. Partial *MGMT* promoter methylation could accurately reflect molecular heterogeneity within the tumor, whereby glioblastomas may be comprised of a heterogeneous mixture of unmethylated and variably hypermethylated tumor cells.[24] For instance, hypermethylated and unmethylated neoplastic cells may segregate to distinct regions of tumor.[25,26] Heterogeneity in the pattern or extent of CpG island methylation has also been described.[6,27] In particular, glioma-initiating cells may be highly enriched for *MGMT* promoter hypermethylation[24] and thus more sensitive to temozolomide therapy.

Previous studies evaluating the clinical significance of partial *MGMT* promoter methylation in glioblastoma patients have generally shown at least some improvement in clinical outcomes following temozolomide therapy compared to their unmethylated *MGMT* promoter counterparts.[14,16–18,28] The extent of methylation may positively correlate with OS,[15,27] although it is unclear if increased *MGMT* promoter methylation past a certain level is associated with additional survival benefit.[17] However, other

studies suggest that partially methylated *MGMT* promoter cases are not associated with better prognosis compared to the unmethylated counterparts[13] and that—when partially methylated *MGMT* promoter cases are combined with hypermethylated cases—the *MGMT* promotion methylation status loses its predictive value.[29] The conflicting literature may be attributable in part to differences in (i) the type of assay and laboratory cutoff values used to determine *MGMT* promoter methylation status and (ii) the clinicopathologic characteristics of the studied patient population.

MGMT promoter methylation status can be tested with several different assays, including (quantitative) methylation specific polymerase chain reaction, pyrosequencing, quantitative real time polymerase chain reaction high resolution melt, methylation-specific multiplex ligation-dependent probe amplification, immunohistochemistry, and genome-wide methylation profiling, each with its own set of benefits and drawbacks.[12] Although certain *MGMT* promoter methylation assays may be better than others at predicting clinical outcome and response to temozolomide therapy in glioblastoma patients,[12,30] there are no consensus guidelines regarding the preferred assay and laboratory cutoff values to determine *MGMT* promoter methylation status.[11] The absence of consensus guidelines is particularly problematic because *MGMT* methylation status may be discordant across assays,[14] and the concordance rate among different laboratories may be as low as 61%.[12] As a step towards standardization of the workup of *MGMT* promoter methylation, Mansouri et al. have outlined a stepwise diagnostic algorithm consisting of inexpensive, widely available, and easily interpretable quantitative methylation specific polymerase chain reaction as an initial test to differentiate overtly hypermethylated from unmethylated glioblastomas, with equivocal *MGMT* promoter methylation cases undergoing reflexive testing using another assay such as pyrosequencing or genome-wide methylation analysis.[12]

Limitations

Our study several has several limitations: (i) the NCDB does not report the methodology or laboratory cutoffs used to determine *MGMT* promoter methylation status, so we cannot account for the variation in assays and cutoff values used in the United States. It is likely that some *MGMT* methylation results that would have been reported as "partially methylated" by one laboratory, may have been dichotomized into either (hyper)methylated or unmethylated by another laboratory. For instance, in a pooled analysis of 4 clinical trials, Hegi et al. classified 10% of glioblastomas as having partially methylated *MGMT* promoters,[16] whereas only 5% of IDH-wildtype glioblastomas were reported as partially methylated in the NCDB. However, because the focus of our investigation was on the outcomes associated with treatment within the partially methylated group, and not between methylation statuses, our analyses should be minimally affected by this limitation. (ii) The NCDB only reports first-line chemotherapy as either none, single-agent, or multi-agent; without further details about specific agents. For IDH-wildtype glioblastoma patients in 2018, we assumed that first-line single-agent chemotherapy coded in the NCDB overwhelmingly represented temozolomide based on its role as the standard-of-care chemotherapeutic agent of choice for IDH-wildtype glioblastoma.[4] No other first-line single-agent regimens were in common clinical use in the United States. It is possible, however, that some patients were treated with other first-line single-agent chemotherapy regimens (eg lomustine monotherapy). (iii) The NCDB does not report the number of cycles, doses, or other details of chemotherapy administration, so we do not know if some patients stopped temozolomide therapy early due to adverse effects or received additional cycles of adjuvant temozolomide. (iv) The NCDB only reports data for a patient's initial diagnosis and first courses of treatment, precluding analysis of therapies administered after the first-line setting.

Conclusions

Our findings provide important insight into the predictive value of partial *MGMT* promoter methylation in newly diagnosed, IDH-wildtype glioblastoma patients. Pertinently, for the practicing oncologist, these data support the use of temozolomide therapy in this patient population and help inform discussions around the prognostic implications of partial methylation when counseling patients. Additional studies are needed to validate standardized workflows for determining *MGMT* methylation status and the laboratory cutoff values that best predict clinical outcome in glioblastoma patients.

Funding

M.T. is supported by the National Cancer Institute of the National Institutes of Health under award number F32CA257210. JBI is supported by the National Cancer Institute of the National Institutes of Health under award number K12CA090354 and Conquer Cancer Foundation/Sontag Foundation.

Conflict of Interest Statement The authors have no conflict of interest to disclose.

Acknowledgments

The National Cancer Data Base (NCDB) is a joint project of the Commission on Cancer (CoC) of the American College of Surgeons and the American Cancer Society. The CoC's NCDB and the hospitals participating in the CoC NCDB are the source of the deidentified data used herein; they have not verified and are not responsible for the statistical validity of the data analysis or the conclusions derived by the authors.

Author Contributions

Conception and study design: JBI. Data collection: JBI. Data analysis: JBI. Data interpretation and Manuscript writing: All authors. Critical review and revisions: All authors.

Data Availability

In accordance with the NCDB data use agreement, data are available by application to the NCDB.

Ethics approval. This study was approved by the Mass General Brigham institutional review board (#2015P002352) and conducted in accordance with the Declaration of Helsinki.

Informed consent. The NCDB Participant User Files contain deidentified national data for which consenting was not applicable.

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