JAMA Oncology | Original Investigation

Association of *MGMT* Promotor Methylation With Survival in Low-grade and Anaplastic Gliomas After Alkylating Chemotherapy

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IMPORTANCE O^6-methylguanine-DNA methyltransferase (*MGMT* [OMIM 156569]) promoter methylation (mMGMT) is predictive of response to alkylating chemotherapy for glioblastomas and is routinely used to guide treatment decisions. However, the utility of *MGMT* promoter status for low-grade and anaplastic gliomas remains unclear due to molecular heterogeneity and the lack of sufficiently large data sets.

OBJECTIVE To evaluate the association of mMGMT for low-grade and anaplastic gliomas with chemotherapy response.

DESIGN, SETTING, AND PARTICIPANTS This cohort study aggregated grade II and III primary glioma data from 3 prospective cohort studies with patient data collected from August 13, 1995, to August 3, 2022, comprising 411 patients: MSK-IMPACT, EORTC (European Organization of Research and Treatment of Cancer) 26951, and Columbia University. Statistical analysis was performed from April 2022 to January 2023.

EXPOSURE *MGMT* promoter methylation status.

MAIN OUTCOMES AND MEASURES Multivariable Cox proportional hazards regression modeling was used to assess the association of mMGMT status with progression-free survival (PFS) and overall survival (OS) after adjusting for age, sex, molecular class, grade, chemotherapy, and radiotherapy. Subgroups were stratified by treatment status and World Health Organization 2016 molecular classification.

RESULTS A total of 411 patients (mean [SD] age, 44.1 [14.5] years; 283 men [58%]) met the inclusion criteria, 288 of whom received alkylating chemotherapy. *MGMT* promoter methylation was observed in 42% of isocitrate dehydrogenase (*IDH*)–wild-type gliomas (56 of 135), 53% of *IDH*-mutant and non-codeleted gliomas (79 of 149), and 74% of *IDH*-mutant and 1p/19q-codeleted gliomas (94 of 127). Among patients who received chemotherapy, mMGMT was associated with improved PFS (median, 68 months [95% CI, 54-132 months] vs 30 months [95% CI, 15-54 months]; log-rank $P < .001$; adjusted hazard ratio [aHR] for unmethylated *MGMT*, 1.95 [95% CI, 1.39-2.75]; $P < .001$) and OS (median, 137 months [95% CI, 104 months to not reached] vs 61 months [95% CI, 47-97 months]; log-rank $P < .001$; aHR, 1.65 [95% CI, 1.11-2.46]; $P = .01$). After adjusting for clinical factors, *MGMT* promoter status was associated with chemotherapy response in *IDH*–wild-type gliomas (aHR for PFS, 2.15 [95% CI, 1.26-3.66]; $P = .005$; aHR for OS, 1.69 [95% CI, 0.98-2.91]; $P = .06$) and *IDH*-mutant and codeleted gliomas (aHR for PFS, 2.99 [95% CI, 1.44-6.21]; $P = .003$; aHR for OS, 4.21 [95% CI, 1.25-14.2]; $P = .02$), but not *IDH*-mutant and non-codeleted gliomas (aHR for PFS, 1.19 [95% CI, 0.67-2.12]; $P = .56$; aHR for OS, 1.07 [95% CI, 0.54-2.12]; $P = .85$). Among patients who did not receive chemotherapy, mMGMT status was not associated with PFS or OS.

CONCLUSIONS AND RELEVANCE This study suggests that mMGMT is associated with response to alkylating chemotherapy for low-grade and anaplastic gliomas and may be considered as a stratification factor in future clinical trials of patients with *IDH*–wild-type and *IDH*-mutant and codeleted tumors.

JAMA Oncol. doi:10.1001/jamaoncol.2023.0990
Published online May 18, 2023.

Invited Commentary
Supplemental content

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Epigenetic silencing of *MGMT* (O^6-methylguanine-DNA methyltransferase [OMIM 156569]) is considered the single most important biomarker predictive of response to temozolomide (TMZ) chemotherapy for glioblastoma (GBM).[1] The *MGMT* gene encodes a DNA-repair protein that removes alkyl groups from the O^6 position of guanine, an important site of DNA alkylation, and, therefore, is believed to confer resistance to alkylating chemotherapy.[2] Conversely, epigenetic silencing of *MGMT* through promoter methylation (mMGMT) is associated with response to treatment.[2-4] Despite widespread acceptance and use of *MGMT* promoter status to guide treatment decisions for GBM,[1,5] its role as a biomarker for low-grade and anaplastic gliomas remains unclear.[6,7] To our knowledge, no randomized clinical trial has yet demonstrated a role for *MGMT* promoter status to guide treatment decisions for grade II and III gliomas.[8-11] Post hoc analyses and nonrandomized prospective studies have yielded mixed results (eTable 1 in Supplement 1),[12-24] in part due to sample sizes that were not large enough to distinguish the molecular heterogeneity among low-grade and anaplastic gliomas.

Since 2016, the World Health Organization (WHO) has redefined gliomas along molecular features, rather than tumor morphologic characteristics.[25] The molecular classification system is defined principally by the presence of an isocitrate dehydrogenase 1 or 2 (*IDH1/2* [*IDH1*, OMIM 147700; *IDH2*, OMIM 147650]) mutation, which is present in more than 70% of gliomas and 5% to 7% of primary GBMs,[26-28] and 1p/19q chromosomal codeletion, which is present in approximately 70% of oligodendrogliomas, 4% of astrocytomas, and 2% of GBMs.[29] Therefore, analyses of historical clinical trials that recruited patients based on histologic features represent mixed populations of patients by current standards. Furthermore, the high frequency of co-occurrence between mMGMT and *IDH* mutation and/or 1p/19q codeletion makes it especially difficult to disentangle the independent association of mMGMT with treatment response.[8,9,14,30-32] To our knowledge, no prospective study to date has found a significant association of *MGMT* methylation with treatment response for *IDH*-mutant glioma after accounting for 1p/19q status.

In this study, we sought to evaluate the association of mMGMT with treatment response for molecularly defined low-grade and anaplastic gliomas. We combined data from 3 prospective glioma cohorts, creating the largest database, to our knowledge, of treated gliomas with both 1p/19q-codeleted and non-codeleted tumors, to date, and stratified analyses based on receipt of alkylating chemotherapy.

Key Points

Question Is *MGMT* promoter methylation associated with response to chemotherapy for molecularly classified low-grade and anaplastic gliomas?

Findings In this cohort study of 411 patients, *MGMT* promoter methylation was independently associated with progression-free and overall survival among patients who received alkylating chemotherapy, specifically among patients with isocitrate dehydrogenase–wild-type or isocitrate dehydrogenase–mutant and 1p/19q-codeleted tumors.

Meaning This study suggests that *MGMT* promoter methylation is a biomarker associated with response to alkylating chemotherapy for low-grade and anaplastic gliomas and may be considered as a stratification factor in future clinical trials.

Methods

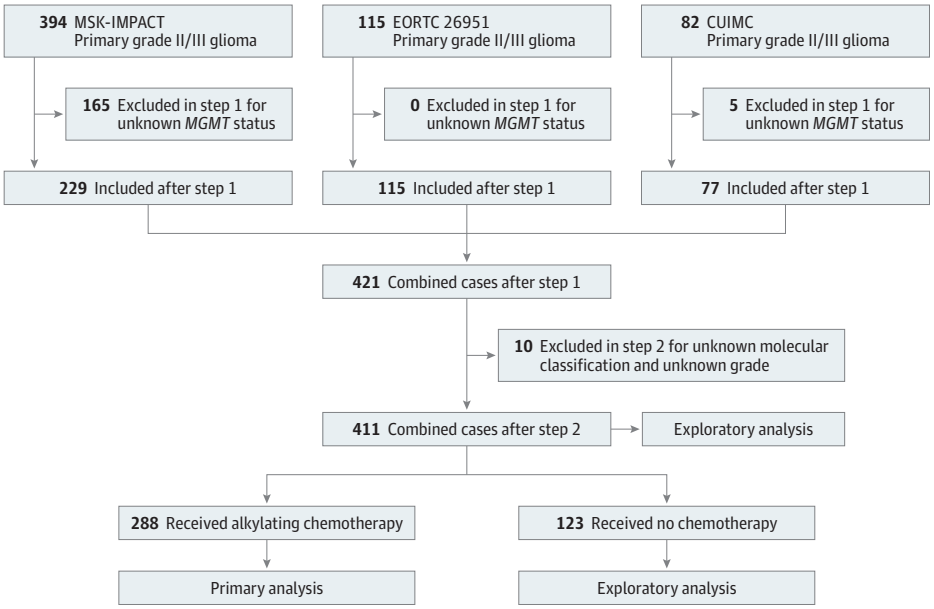
Data Sources

Data sources were identified and selected if the source was derived from a prospective study, included information on mMGMT status and survival status, and contained individual patient-level data. We identified 3 available studies, with patient data collected from August 13, 1995, to August 3, 2022, that met these criteria. The study by Jonsson et al[33] (MSK-IMPACT) was a prospective study conducted at Memorial Sloan Kettering Cancer Center designed to integrate genomic data with clinical and treatment phenotypes to assess genetic aberrations in glioma and GBM that are associated with clinical behavior, evolution of therapy, or response to therapy. A randomized clinical trial designed by the European Organization of Research and Treatment of Cancer, EORTC 26951, evaluated the effectiveness of adjuvant alkylating chemotherapy for anaplastic oligodendroglial tumors.[34] A third cohort was derived from the Columbia University Irving Medical Center (CUIMC) for patients prospectively enrolled in the Comprehensive Brain Malignancy, Brain Tumor and Brain Radiotherapy Clinical Database. Detailed descriptions of the source data and variable selection and coding are available in the eMethods in Supplement 1. Data on patients treated at CUIMC in the Department of Radiation Oncology were enrolled to the Comprehensive Brain Malignancy, Brain Tumor and Brain Radiotherapy Clinical Database; these patients provided written consent. Data collection on deidentified publicly available databases (MSK-IMPACT and EORTC 26951) were exempt from review by the Columbia University institutional review board. This report followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for observational studies.

Variable Selection and Coding, Patient Selection, and Outcomes

Age, sex, molecular classification, tumor grade, chemotherapy, radiotherapy, and *MGMT* promoter methylation status were included as covariables. Patients were included in the study if they received a diagnosis of a primary grade II or III glioma (astrocytoma, oligodendroglioma, or oligoastrocytoma histologic characteristics). Patients were excluded if they had unknown *MGMT* promoter status, could not be classified by *IDH* and 1p/19q codeletion status, had unknown histologic grade, or were previously treated with chemotherapy or radiotherapy. The primary outcome was progression-free survival (PFS), and the secondary outcome was overall survival (OS).

Figure 1. Schema for Patient Selection and Analyses



CUIMC indicates Columbia University Irving Medical Center; and EORTC, European Organization of Research and Treatment of Cancer.

Statistical Analysis

Statistical analysis was performed from April 2022 to January 2023. All statistical analyses were conducted using RStudio software, version 1.4.1106 (RStudio Inc). For our primary analysis, we measured the association between *MGMT* promoter status and PFS or OS among patients who received chemotherapy, stratified by molecular classification and adjusted for covariables. Exploratory analyses included PFS and OS for all patients, regardless of treatment, and for patients who did not receive chemotherapy (**Figure 1**). Descriptive statistics were generated and stratified by *MGMT* promoter status. Associations between *MGMT* promoter status and other clinical variables were determined using the Pearson χ^2 test and the Wilcoxon rank sum test. The Fisher exact test was substituted for comparisons with expected frequency cell counts less than 5. Kaplan-Meier estimates were obtained for PFS and OS and compared between *MGMT* promoter status using the log-rank test. Univariable and multivariable Cox proportional hazards regression analyses of both PFS and OS were conducted. Variables that approached significance (using $P < .15$ as cutoff) were included in the multivariable analysis. Interactions between *MGMT* promoter status and chemotherapy were tested within the univariable and multivariable Cox proportional hazards regression models. The Schoenfeld test of weighted residuals was used to assess proportional hazard assumptions.[35] If the proportional hazards assumptions for a variable were violated, the variable was removed and used as a stratification factor in the Cox proportional hazards regression models (as a sensitivity analysis). The Benjamini-Hochberg method was used to adjust for multiple hypothesis testing in molecular subgroups for the primary and secondary outcomes. All analyses were performed at the .05 significance level based on 2-sided statistical testing.

Results

Demographic and Clinical Characteristics

We identified 591 patients with primary grade II or III glioma, 411 (70%; mean [SD] age, 44.1 [14.5] years; 283 men [58%]) of whom met inclusion criteria with known *MGMT* promoter status, *IDH* and 1p/19q codeletion status, and tumor grade (Figure 1). Clinical and demographic characteristics are displayed in **Table 1**. A total of 288 patients (70%) received chemotherapy as first-line treatment before progression. Of the patients who received chemotherapy, 226 (79%) received TMZ-based regimens; 61 (21%) received procarbazine, lomustine, and vincristine (PCV); and 1 (0.3%) received carmustine (eTable 2 in Supplement 1). The *MGMT* promoter was methylated in 42% of *IDH*-wild-type gliomas (56 of 135), 53% of *IDH*-mutant and non-codeleted gliomas (79 of 149), and 74% of *IDH*-mutant and codeleted gliomas (94 of 127) ($P < .001$). Patients with mMGMT were more likely to receive radiotherapy (59% [135 of 229] vs 48% [87 of 182]; $P = .02$). The median follow-up time was 40 months (IQR, 16-84 months), with 242 progressions (59%) and 177 deaths (43%). A summary of clinical characteristics stratified by molecular class is displayed in eTables 3, 4, and 5 in Supplement 1.

Survival Analysis of Patients Receiving Alkylating Chemotherapy

Our primary analysis included patients who received alkylating chemotherapy as part of their initial course of treatment before progression (Figure 1). The results are summarized in **Table 2**. Full univariable and multivariable analyses are available in eTables 6 to 13 in Supplement 1.

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	No. (%)		*P* value[a]
	Methylated *MGMT* promoter (n = 229)	Unmethylated *MGMT* promoter (n = 182)	
Age, median (IQR), y	43 (35-53)	44 (31-56)	.69
Sex			
Female	96 (42)	77 (42)	.94
Male	133 (58)	105 (58)	
Molecular class			
IDH-wild type	56 (25)	79 (43)	<.001
IDH-mutant and non-codeleted	79 (35)	70 (39)	
IDH-mutant and codeleted	94 (41)	33 (18)	
Grade			
II	78 (34)	72 (40)	.25
III	151 (66)	110 (60)	
Chemotherapy			
Yes	157 (69)	131 (72)	.45
No	72 (31)	51 (28)	
Radiotherapy			
Yes	135 (59)	87 (48)	.02
No or unknown[b]	94 (41)	95 (52)	

[a] Calculated with the Pearson χ^2 test and the Wilcoxon rank sum test.

[b] For patients in the MSK-IMPACT data set, receipt of radiotherapy was inferred from subsequent surgical samples. Therefore, if no further surgeries were conducted, the patient was coded as "No or unknown" (eMethods in Supplement 1).

Table 2. Summary of Univariable and Multivariable Subgroup Analyses in Patients Who Received Chemotherapy[a]

Molecular subgroup	Univariable			Multivariable[b]		
	HR of uMGMT vs mMGMT (95% CI)	*P* value	*P* value for interaction with treatment[b]	Adjusted HR of uMGMT vs mMGMT (95% CI)	*P* value	*P* value for interaction with treatment (adjusted)[c]
Progression-free survival						
All cases	2.29 (1.66-3.17)	<.001	.004	1.95 (1.39-2.75)	<.001[d]	.004
IDH-wild type	1.95 (1.15-3.30)	.01	.004	2.15 (1.26-3.66)	.005[d]	<.001
IDH-mutant and non-codeleted	1.19 (0.67-2.12)	.56	.41	NR[e]	NR[e]	.94
IDH-mutant and codeleted	2.54 (1.24-5.20)	.01	.39	2.99 (1.44-6.21)	.003[d]	.59
Overall survival						
All cases	2.30 (1.57-3.37)	<.001	.003	1.65 (1.11-2.46)	.01[d]	.01
IDH-wild type	1.83 (1.06-3.15)	.03	.12	1.69 (0.98-2.91)	.06	.02
IDH-mutant and non-codeleted	1.07 (0.54-2.12)	.85	.32	NR[e]	NR[e]	.70
IDH-mutant and codeleted	2.40 (0.81-7.17)	.12	.21	4.21 (1.25-14.2)	.02[d]	.26

Abbreviations: HR, hazard ratio; mMGMT, methylated *MGMT* promoter; NR, not reported; uMGMT, unmethylated *MGMT* promoter.

[a] Summary statistics are provided from separate univariable and multivariable analyses in patients who received alkylating chemotherapy and stratified analyses by molecular subgroups. Full univariable and multivariable analyses are included in eTables 6, 7, 8, 9, 10, 11, 12, and 13 in Supplement 1. Multiple hypothesis testing in molecular subgroups was accounted for in the multivariable analyses of progression-free and overall survival.

[b] Variables included in the analysis were age, sex, molecular class, grade, chemotherapy, and radiotherapy. Variables that approached significance on univariable analysis, using $P < .15$ as a threshold, were included in the multivariable analyses.

[c] Interaction between *MGMT* promoter status and chemotherapy was derived from analysis for all patients stratified by the chemotherapy and no chemotherapy groups (Figure 1; eTable 17 in Supplement 1).

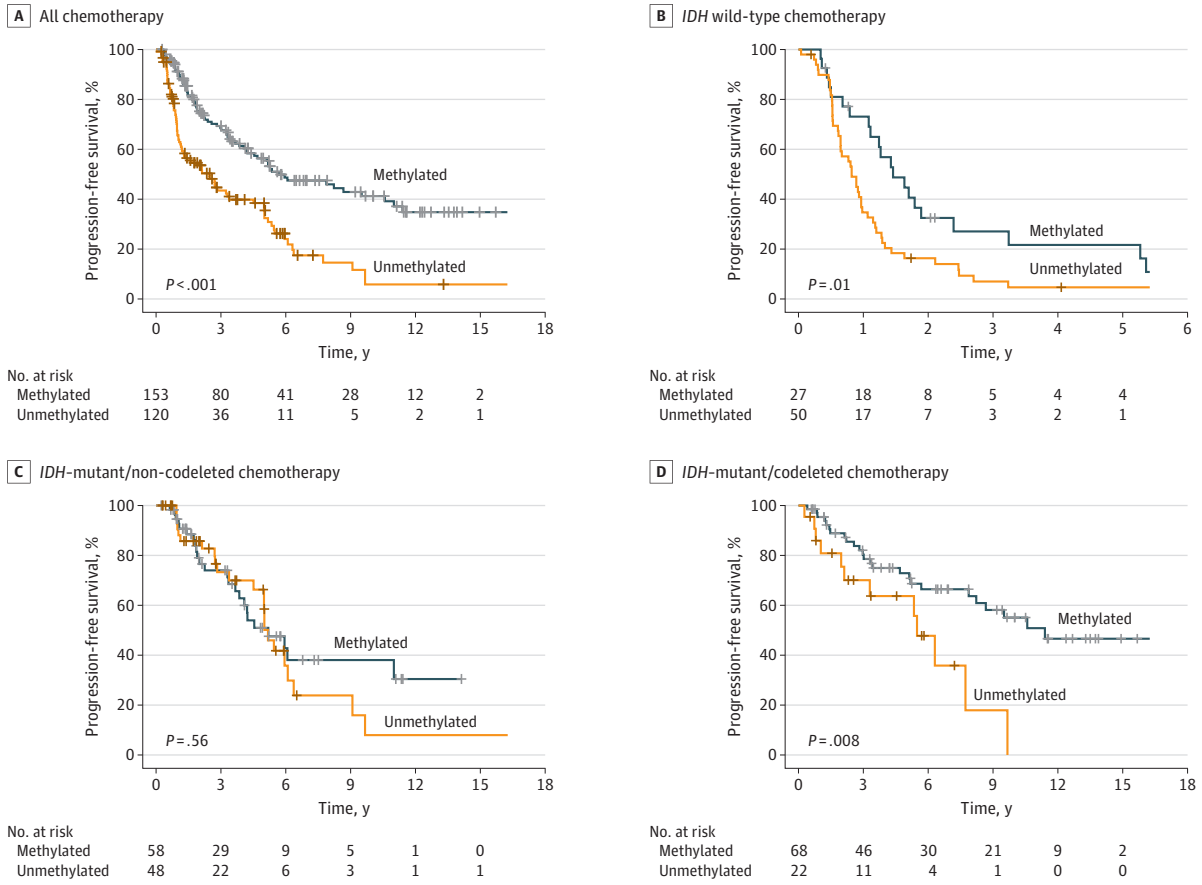
[d] Significant after adjusting for multiple hypothesis testing.

[e] Variables that were not significant on univariable analysis were not included in the multivariable analysis and therefore not reported.

Among patients who received alkylating chemotherapy, the median PFS was 54 months (95% CI, 40-64 months), and the median OS was 92 months (95% CI, 77-120 months). The median PFS and OS were significantly longer among patients with tumors with mMGMT vs those with tumors with an unmethylated *MGMT* promoter (uMGMT) (PFS, 68 months [95% CI, 54-132 months] vs 30 months [95% CI, 15-54 months]; $P < .001$; OS, 137 months [95% CI, 104 months to not reached [NR]] vs 61 months [95% CI, 47-97 months]; $P < .001$) (**Figure 2** and **Figure 3**). After adjustment for age, sex, molecular class, grade, and receipt of radiotherapy, *MGMT* promoter status remained significantly associated with survival (adjusted hazard ratio [aHR] of u*MGMT* for PFS, 1.95 [95% CI, 1.39-2.75]; $P < .001$; and OS, 1.65 [95% CI, 1.11-2.46]; $P = .01$) (Table 2).

We further stratified our analysis by molecular class. The median PFS times were 12 months (95% CI, 9.6-15 months) for *IDH*-wild-type tumors, 62 months (95% CI, 54-76 months) for *IDH*-mutant and non-codeleted tumors, and 114 months (95%

Figure 2. Kaplan-Meier Curves for Progression-Free Survival Based on *MGMT* Promoter Methylation Status



A, Patients who received chemotherapy. B, Patients with *IDH*-wild type tumors. C, Patients with *IDH*-mutant and non-codeleted tumors. D, Patients with *IDH*-mutant and codeleted tumors.

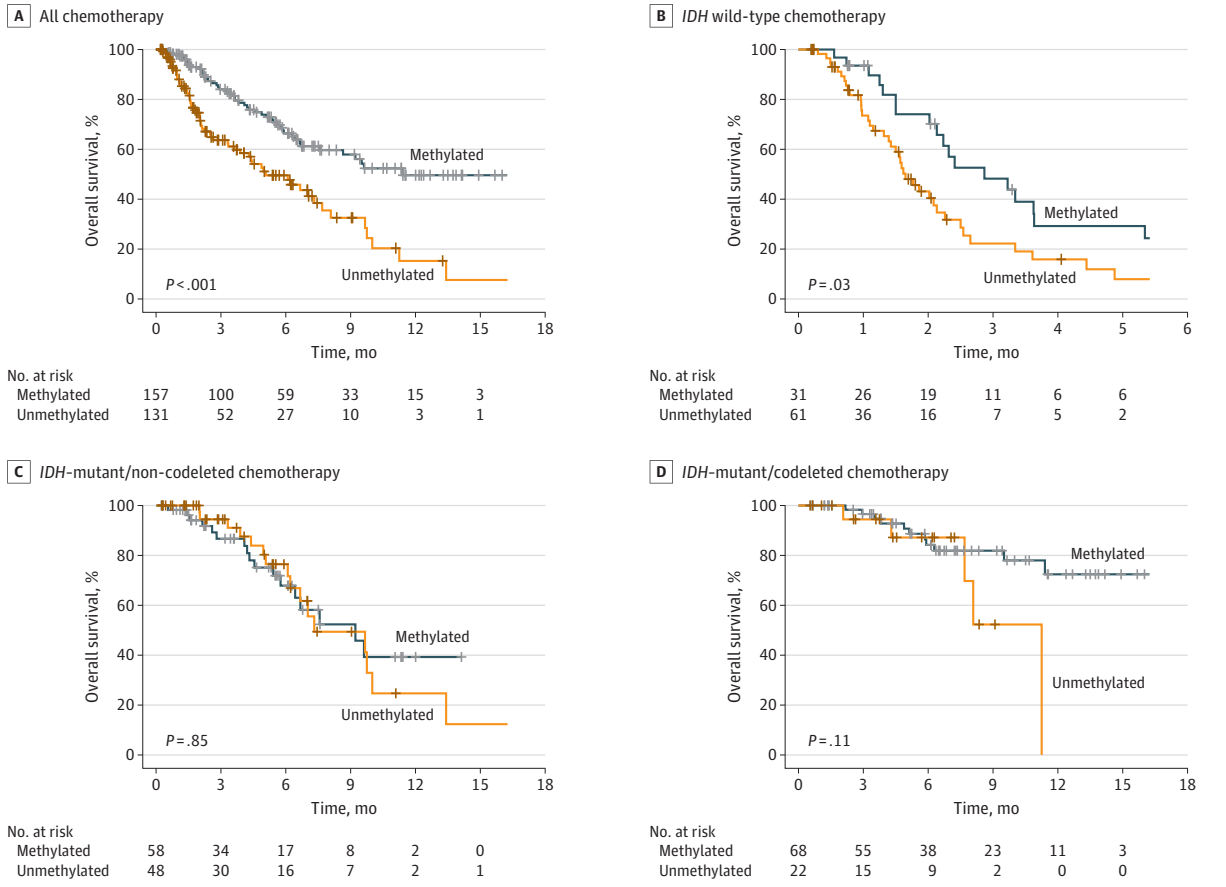
CI, 76 months to NR) for *IDH*-mutant and codeleted tumors. The median OS times were 25 months (95% CI, 19-30 months) for *IDH*-wild-type tumor, 111 months (95% CI, 80-NR) for *IDH*-mutant and non-codeleted tumors, and 253 months (95% CI, 137-NR) for *IDH*-mutant and codeleted tumors. For *IDH*-wild-type tumors, mMGMT status was associated with improved median PFS compared with uMGMT status (18 months [95% CI, 13-39 months] vs 9.8 months [95% CI, 7.8-13 months]; $P = .01$; aHR for uMGMT, 2.15 [95% CI, 1.26-3.66], $P = .005$) and improved median OS compared with uMGMT status (34 months [95% CI, 26-67 months] vs 20 months [95% CI, 17-27 months]; $P = .03$). However, the association with OS was not significant on multivariable analysis (aHR for uMGMT, 1.69 [95% CI, 0.98-2.91]; $P = .06$). For *IDH*-mutant and codeleted tumors, mMGMT status was independently associated with PFS compared with uMGMT status (median, 11.4 years [95% CI, 8.25-NR] vs 5.5 years [95% CI, 40-NR]; $P = .008$; aHR for uMGMT, 2.99 [95% CI, 1.44-6.21]; $P = .003$). In univariable analysis, mMGMT status was not associated with OS for *IDH*-mutant and codeleted tumors compared with uMGMT status (median, 21.1 years [95% CI NR-NR] vs 11.3 years [95% CI, 7.7-NR]; $P = .11$), but mMGMT status was independently associated with OS after accounting for other known confounders (aHR for uMGMT, 4.21 [95% CI, 1.25-14.2]; $P = .02$).

Among *IDH*-mutant and non-codeleted tumors, mMGMT status was not associated with PFS compared with uMGMT status (median, 62 months [95% CI, 46-NR] vs 62 months [95% CI, 60-109 months; $P = .56$; aHR for uMGMT, 1.19 [95% CI, 0.67-2.12]; $P = .56$) or OS (median, 111 months [95% CI, 77-NR] vs 88 months [95% CI, 80-NR]; $P = .85$; aHR for uMGMT, 1.07 [95% CI, 0.54-2.12]; $P = .85$).

Interaction With Treatment

We conducted exploratory analyses for all patients, regardless of chemotherapy status, and for patients who did not receive alkylating chemotherapy before progression (Figure 1). Among all patients, the association of methylation was significant but attenuated for PFS (aHR, 1.36 [95% CI, 1.04-1.79]; $P = .03$) and was no longer significant for OS (aHR, 1.17 [95% CI, 0.86-1.60]; $P = .31$) (eTable 14 and eFigure 1 in Supplement 1). The effect size of methylation was attenuated for *IDH*-mutant and codeleted tumors and was no longer significant for *IDH*-wild-type tumors. Among patients who did not receive chemotherapy, mMGMT was not associated with poorer

Figure 3. Kaplan-Meier Curves for Overall Survival Based on *MGMT* Promoter Methylation Status



A, Patients who received chemotherapy. B, Patients with *IDH*-wild-type tumors. C, Patients with *IDH*–mutant and non-codeleted tumors. D, Patients with *IDH*–mutant and codeleted tumors.

PFS or OS in any subgroup and was associated with improved PFS in *IDH*-wild-type tumors (aHR, 0.42 [95% CI, 0.21-0.85]; *P* = .02) (eTable 15 and eFigure 2 in Supplement 1). There was a significant interaction between mMGMT status and chemotherapy for the entire cohort (Table 2).

Sensitivity Analyses

There were 2 patients with anaplastic oligodendrogliomas in the EORTC 26951 cohort with discordant *IDH1* and 1p/19q codeletion status (*IDH1*-wild type and 1p/19q codeleted).[36] *IDH2* mutational status, which accounts for approximately 10% of *IDH*-mutant anaplastic astrocytomas, was not available for this cohort.[37] Additional molecular markers, such as *ATRX* and *TP53*, were not available to resolve this discordance.[38] Because false-positive 1p/19q codeletions detected via fluorescent in situ hybridization are rare in properly worked-up samples,[38] we assumed that these 2 cases were *IDH*-mutant and codeleted tumors. However, we performed a sensitivity analysis in which these 2 cases were coded as *IDH*-wild-type tumors with similar results observed (eTable 16 in Supplement 1).

In the primary analysis of PFS among patients who received chemotherapy, the proportional hazards assumption was violated for molecular class and grade (eFigures 3 and 4 in Supplement 1). Stratification by molecular class was conducted as a part of the primary analysis (Figure 2; Table 2). After stratification by molecular status, age and grade in the *IDH*-wild-type subgroup and grade in the *IDH*-mutant and non-codeleted subgroup violated the proportional hazards assumption. Nonetheless, similar results were found after stratifying our analysis by these factors (eFigure 5 and eTables 17-21 in Supplement 1). In our secondary analysis of OS among patients who received chemotherapy, the proportional hazards assumption was violated for age, molecular class, and grade (eFigures 6 and 7 in Supplement 1). There were no violations of the proportional hazards assumption after stratifying by molecular class.

Because there were only 17 deaths in the *IDH*-mutant and codeleted group, we performed a sensitivity analysis including only covariables that were statistically significant at the *P* < .05 threshold, to account for potential overfitting of the model. Similar results were observed (eTable 22 in Supplement 1). Performance status and extent of resection were available for 185 patients in the EORTC and CUIMC cohorts. Methylated *MGMT* promoter status was not associated with performance status or extent of resection among patients with

IDH-wild-type and *IDH*-mutant and codeleted tumors and was associated with PFS and OS after chemotherapy in a multivariable model that included performance status and extent of resection (eFigure 8 and eTables 23-27 in Supplement 1).

Discussion

In this study, we found that mMGMT was associated with response to alkylating chemotherapy for low-grade and anaplastic gliomas. Specifically, *MGMT* status was associated with response to treatment for 1p/19q-codeleted and *IDH*-wild-type tumors in patients who received alkylating chemotherapy, but not for *IDH*-mutant and non-codeleted tumors, regardless of treatment status. To our knowledge, this is the first study to report an independent association of *MGMT* methylation with response to treatment for low-grade and anaplastic gliomas after accounting for both *IDH* and 1p/19q codeletion status, and it is the only study to report the association of *MGMT* methylation with response to treatment in 1p/19q-codeleted tumors.

Radiotherapy with neoadjuvant or adjuvant PCV is a standard therapy for anaplastic oligodendroglial tumors based on the RTOG 9402 and EORTC 26951 trials, particularly for tumors with *IDH* mutations and/or 1p/19q codeletions.[14,39] Although *IDH* mutations and/or 1p1/9q codeletions have been proposed as a biomarker for benefit from treatment with PCV, our ability to isolate the association of *MGMT* status with response to treatment for *IDH*-mutant and 1p/19q-codeleted tumors suggests that *MGMT* status may be a better clinical biomarker for oligodendroglial tumors that are defined by modern molecular guidelines.[15]

This finding is particularly salient in the context of the ongoing CODEL (NCT00887146) and POLCA (NCT02444000) phase 3 clinical trials, which evaluate various alkylating strategies for 1p/19q-codeleted tumors. Given the favorable prognosis of patients with 1p/19q-codeleted gliomas, there is interest in de-escalating therapies, either with substitution of PCV with TMZ or potentially omission of radiotherapy, which are under investigation.

Despite the absence of randomized clinical trials supporting the use of TMZ over PCV and some data suggesting inferior outcomes, TMZ remains commonly used as first-line therapy in clinical practice due to its favorable hematologic toxicity profile.[7,11,40,41] The initial study design of the phase 3 CODEL trial randomized patients with anaplastic 1p/19q-codeleted gliomas to receive radiotherapy alone, TMZ alone, or radiotherapy with concomitant and adjuvant TMZ.[11] Analysis of the first 36 patients randomized suggested inferior PFS for patients treated with TMZ alone vs either of the radiotherapy groups. The CODEL trial was subsequently modified, and its current design compares radiotherapy plus TMZ in the experimental group with radiotherapy plus PCV in the standard group. Although *MGMT* status was not included as a stratification factor, correlation between exploratory biomarkers (ie, *MGMT* status) and survival is a prespecified secondary goal.

The phase 3 POLCA trial, currently enrolling patients in France, similarly seeks to de-escalate therapy for anaplastic 1p/19q-codeleted gliomas, testing whether omission of radiotherapy with PCV alone can improve neurocognitive deterioration without decreasing PFS and OS compared with standard-of-care radiotherapy plus PCV. Although the POLCA trial does not prespecify prospective analysis of *MGMT* methylation as a study goal, conclusions from our study suggest that *MGMT* promoter status may be evaluated in a post hoc manner when available. The CODEL and POLCA trials are expected to complete enrollment in 2025 and 2024, respectively; therefore, results will not be available for many years.

IDH-wild-type tumors, in contrast to *IDH*-mutant tumors, have more consistently demonstrated an association between *MGMT* methylation and response to treatment.[17,18,20,23] Our results largely validate findings from the recently published CATNON trial, which found *MGMT* status to be prognostic in anaplastic *IDH*-wild-type gliomas.[23] In the CATNON trial, there was no benefit associated with concurrent or adjuvant TMZ to radiotherapy in an unplanned subgroup analysis of patients with *IDH*-wild-type tumors. When the study group further defined a cohort of molecular GBMs in 157 of 751 patients randomized (defined by telomerase reverse transcriptase promoter mutation, chromosome 7 trisomy and loss of heterozygosity of chromosome 10, or epidermal growth factor receptor amplification), *MGMT* was prognostic, but concurrent or adjuvant TMZ was not associated with benefit in both the mMGMT and uMGMT subgroups.[24] Although this post hoc analysis of the CATNON trial is insufficient to warrant a change in standard-of-care treatment, the authors stated that a well-powered prospective study on the effectiveness of TMZ in *IDH*-wild-type gliomas is warranted. *MGMT* status may be considered as a stratification factor, in the same way as it has been done in contemporary GBM trials defined by histologic and molecular characteristics.[3,4,42]

In the CATNON trial, *IDH*-mutant and non-codeleted tumors benefited from adjuvant but not concurrent TMZ, which defines a new standard of care.[10,23,43] Consistent with our findings, *MGMT* status was not associated with response to TMZ in *IDH*-mutant and non-codeleted tumors. The interaction between *MGMT* and codeletion status that we demonstrated in our study may resolve the discordant results observed from previous studies, which inconsistently found *MGMT* status to be associated with response to treatment in *IDH*-mutant tumors but did not simultaneously account for 1p/19q codeletion status (eTable 1 in Supplement 1).[9,13-15,17-20,22] Our results emphasize that *IDH*-mutant and codeleted and non-codeleted tumors may be considered separate entities with regard to the association of mMGMT with response to treatment.

In the most recent update of the WHO Classification of Tumors of the Central Nervous System, published in November 2021, *MGMT* status does not currently change grading of gliomas or GBMs but may be incorporated into future updates.[44] Challenges in the standardization of laboratory assays to detect mMGMT hinders widespread adoption in clinical practice. There is considerable variability between techniques, and it has been shown that specific methylation sites on CpG islands within the *MGMT* promoter may be more associated with response to treatment than others.[15] The most recent clinical

trials have used a methylation-specific polymerase chain reaction technique with the STP27 algorithm.[9,10,15,16,20,22]

Strengths and Limitations

Our study has some strengths, including analysis of prospectively enrolled patients with high-quality data. With the exception of the recently published CATNON second interim analysis, this is one of the largest studies of *MGMT* methylation in prospectively collected low-grade and anaplastic gliomas and the largest of 1p/19q-codeleted gliomas, to our knowledge.

Our study also has some limitations, including retrospective or post hoc analysis of prospectively enrolled patients. Treatments and follow-up are heterogenous, and thus unmeasured confounders may have affected our results.[8,34,45,46] Definitions of progression may have varied slightly across the data sets. Radiotherapy was more commonly delivered to patients with methylated tumors, likely because radiotherapy was the standard group in the EORTC study of anaplastic oligodendroglial tumors. Given that *MGMT* status is not routinely incorporated into treatment-related decisions for low-grade and anaplastic gliomas, it is unlikely that clinician knowledge of *MGMT* status, when applicable, was associated with treatment choices. Finally, because deaths were rare for patients with 1p/19q-codeleted tumors, the association of *MGMT* status with OS depended on the statistical parameters used. Validation with longer follow-up would support that the PFS advantage observed translates into an OS benefit. Because we are the first to report an association of mMGMT with treatment response for 1p/19q-codeleted tumors, our work should be externally validated.

Conclusions

Our cohort study shows that *MGMT* status was associated with response to alkylating chemotherapy for molecularly classified low-grade and anaplastic gliomas. Specifically, it was associated with response to chemotherapy among patients with *IDH*-wild-type or *IDH*-mutant and codeleted tumors. Therefore, *MGMT* status may be considered a stratification factor in future clinical trials evaluating the effectiveness of alkylating chemotherapy. This may be particularly relevant for *IDH*-wild-type and *IDH*-mutant and codeleted tumors, in which the role of TMZ and/or PCV in standard-of-care treatment remains obscure and an area of active investigation.

ARTICLE INFORMATION

Accepted for Publication: February 13, 2023.

Published Online: May 18, 2023. doi:10.1001/jamaoncol.2023.0990

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Conflict of Interest Disclosures: Dr McKhann reported receiving personal fees from Koh Young Inc and NeuroOne Medical Technologies; and grants from the National Institutes of Health, outside the submitted work. Dr Iwamoto reported receiving personal fees from Mimivax, Praesidia Biotherapeutics, Roche, Novocure, Guidepoint, Sapience, XCures, Abbvie, Alexion, Gennao Bio, Medtronic, Regeneron, and Tocagen; and grants from Glioblastoma Foundation, Rhodes Brain Tumor Center, and Ivy Foundation, outside the submitted work. Dr Kachnic reported receiving grants from Varian Inc; personal fees from New Beta Innovation Limited; serving as editor of the Bones Metastasis chapter for, holding licenses from, and receiving royalties from UpToDate; and receiving nonfinancial support from Radiation Therapy Oncology Group Foundation outside the submitted work. Dr Yu reported receiving personal fees from Boston Scientific, Myovant, and Reflexion Medical; and grants from Pfizer, outside the submitted work. Dr Wang report receiving personal fees and nonfinancial support from AbbVie, Elekta, Merck, Novocure, and the RTOG Foundation; personal fees from Cancer Panels, Doximity, Rutgers, University of Iowa, Wolters Kluwer, and Iylon Precision Oncology; and grants and nonfinancial support from Genentech and Varian, outside the submitted work. No other disclosures were reported.

Funding/Support: This study was supported by grant P30 CA13696 from the National Cancer Institute.

Role of the Funder/Sponsor: The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Meeting Presentation: This study was presented at the Annual Meeting of the American Society for Radiation Oncology; October 25, 2021; San Antonio, Texas.

Data Sharing Statement: See Supplement 2.

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