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Dual *MGMT* inactivation by promoter hypermethylation and loss of the long arm of chromosome 10 in glioblastoma

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Abstract

Background: Epigenetic inactivation of O6-methylguanine-methyltransferase (*MGMT*) gene by methylation of its promoter is predictive of Temozolomid (TMZ) response in glioblastoma (GBM). *MGMT* is located on chromosome 10q26 and the loss of chromosome 10q is observed in 70% of GBMs. In this study, we assessed the hypothesis that the dual inactivation of *MGMT*, by hypermethylation of *MGMT* promoter and by loss the long arm of chromosome 10 (10q), may confer greater sensitivity to TMZ.

Methods: A total of 149 tumor samples from patients diagnosed with GBM based on the WHO 2016 classification were included in this retrospective study between November 2016 and December 2018. Methylation status of *MGMT* promoter was evaluated by pyrosequencing and status of chromosome 10q was assessed by array comparative genomic hybridization.

Results: Glioblastoma patients with chromosome 10q loss associated with hypermethylation of *MGMT* promoter had significantly longer overall survival (OS) ($P = .0024$) and progression-free survival (PFS) ($P = .031$). Indeed, median OS of patients with dual inactivation of *MGMT* was 21.5 months compared to 12 months and 8.1 months for groups with single *MGMT* inactivation by hypermethylation and by 10q loss, respectively. The group with no *MGMT* inactivation had 9.5 months OS. Moreover, all long-term survivors with persistent response to TMZ treatment (OS $\geq$ 30 months) displayed dual inactivation of *MGMT*.

Conclusions: Our data suggest that the molecular subgroup characterized by the dual inactivation of *MGMT* receives greater benefit from TMZ treatment. The results of our study may be of immediate clinical interest since chromosome 10q status and methylation of *MGMT* promoter are commonly determined in routine practice.

KEYWORDS

10q, comparative genomic hybridization, glioblastoma, loss of heterozygosity, MGMT

1 | INTRODUCTION

Glioblastoma (GBM) is the most common and aggressive malignant primary tumor of the central nervous system (CNS) in adults.[1,2] The therapeutic standard (Stupp's protocol) is currently defined by maximal safe surgical resection followed by radiotherapy plus concomitant alkylating agent temozolomide (TMZ) followed by adjuvant chemotherapy with TMZ.[3-5] However, the response to TMZ varies from one patient to another.[6]

Epigenetic silencing of *MGMT* (*O6-Methylguanine-DNA methyltransferase*) by promoter methylation is common in GBM (40%-50%).[7] It is predictive of the therapeutic response to alkylating agents such as TMZ, and therefore associated with patient survival.[8,9] *MGMT* encodes for DNA repair protein, which removes the alkyl groups at the O6-guanine position induced by alkylating agents. As a result, when not silenced, MGMT neutralizes TMZ cytotoxic action by reducing its therapeutic effect.[10]

The *MGMT* is located at chromosome 10q26.3 and loss of chromosome 10q is frequently observed in GBM (70%).[11] Despite the importance of 10q loss in gliomagenesis, its association with survival remains controversial. Numerous trials have studied the prognostic impact of 10q loss in GBMs and reported either negative[12-15] or neutral[16-20] impact on survival.

In tumor cells, the loss of chromosome 10q26.3 implies a loss of heterozygosity (LOH) of *MGMT*. If the promoter of *MGMT* carried by the second allele is hypermethylated, in theory the tumor cells present complete silencing of *MGMT* gene expression. This GBM molecular subtype may present greater sensitivity to TMZ than GBM with *MGMT* inactivation by a single mechanism.

In our study, we aimed to investigate overall survival (OS) and progression-free survival (PFS) in GBM with dual inactivation of *MGMT* (by methylation of its promoter and chromosome 10q26.3 loss) versus simple inactivation of *MGMT* (by one of the previously cited mechanisms).

2 | MATERIALS AND METHODS

2.1 | Study design

We conducted a retrospective study of tumor samples from patients with GBM originating in six different French hospitals. Tumor samples received for routine exploration at the Cancer Biology Department of Poitiers University Hospital between November 2016 and December 2018 were included in this study.

2.2 | Patients

The study was carried out in accordance with French legislation (French bioethics law No. 2004-800 of 6 August 2004 and Law No 2012-300 of 5 March 2012 on research involving the human person) and in accordance with the Helsinki Declaration. Data confidentiality was ensured for all patients.

The study included 149 patients aged ≥ 18 years with confirmed GBM diagnosis by experienced neuropathologists according to the WHO 2016 CNS classification (Table 1). All tumor samples were available for comparative genomic hybridization (CGH) assay and pyrosequencing analysis and 90% of them presented adequate percentage of tumor cells (above the optimum rate of 50%). As our minimum percentage of tumor cells for these techniques was 20%, the remaining samples with rates between ≥ 20% and < 50% were not excluded from the study. One hundred and forty-two GBM tumors were wild type for *isocitrate dehydrogenase 1/2 genes* (*IDH1/2*), six (4%) were *IDH1* p.R132H-mutated, and one (0.7%) was *IDH2* p.R172K-mutated. General features of the cohort such as age, WHO performance status, were collected from the clinical chart.

2.3 | Treatment and follow-up

Every patient in the study received the recommended standard treatment (Stupp's protocol).[3,4] Tumor progression was determined based on magnetic resonance imaging according to the RANO criteria.[21] Tumor progression management and second-line treatment (surgery, radiotherapy and/or chemotherapy) were discussed in multidisciplinary coordination meetings.

2.4 | Pyrosequencing

All molecular analyses were conducted as routine practice for GBM biomarker testing at the Cancer Department of Poitiers University Hospital (France). Tumor DNA was extracted using the Maxwell® FFPE Tissue LEV DNA kit (AS1130, Promega) from an average of six sections of 10 μm thick fixed paraffin-embedded tissues.

The methylation profile of five CpG sites, located in the region of + 17 to + 39 of exon 1 of the *MGMT* gene (chromosome 10q26 ranging from 131 265 5007 to 131 265 535) was analyzed. The exact sequence was: 5′-**CG**GACAG**CG**ATCTCTAA**CGCG**CAAG**CG**CA-3′. In each series, internal quality control groups were systematically added: a blank and two controls, one highly methylated (MethylatedHuman Control, Promega) and the other unmethylated (UnmethylatedHuman Control DNA, Qiagen). The tumor DNA was bisulfite-modified using the EZ DNA Methylation-Gold kit (ZymoResearchn). PCR amplification was performed using 5 μL of bisulfite-modified DNA using the Pyromark Q24 CpG MGMT® kit (Qiagen) with 1 μL of sense and antisense sequencing primer.

TABLE 1 Demographic, histological, and biological characteristics of patients at inclusion

Features	Group1 (N = 41)	Group2 (N = 27)	Group3 (N = 54)	Group4 (N = 27)	Total (N = 149)	P
Demographic data						
Age at diagnosis (y)						.62
Mean	64	62	63	66	64	
Extreme	26-84	18-88	32-82	31-82	18-88	
Gender—n (%)						.18
Male	18 (44)	17 (63)	35 (65)	17 (63)	87 (58)	
Female	23 (56)	10 (37)	19 (35)	10 (37)	62 (42)	
WHO performance status—n (%)						.91
0	10 (24)	6 (22)	15 (28)	5 (18)	36 (24)	
1	23 (56)	15 (56)	28 (52)	19 (70)	85 (57)	
2	6 (15)	3 (11)	7 (13)	1 (4)	17 (11)	
3	2 (5)	1 (4)	3 (6)	1 (4)	7 (5)	
4	0	1 (4)	1 (2)	0	2 (1)	
Unknown	0	1 (4)	0	1 (4)	2 (1)	
Histological/Biological data						
Type of specimen—n (%)						.35
Biopsy	16 (39)	16 (59)	22 (41)	13 (48)	67 (45)	
Surgical specimen	25 (61)	11 (41)	32 (59)	14 (52)	82 (55)	
2016 WHO classification—n (%)						.47
GBM *IDH* wild type	38 (93)	25 (92.6)	52 (96)	27 (100)	142 (95)	
GBM *IDH* mutated	3 (7)	2 (7)	2 (4)	0	7 (5)	
MGMT methylation						**<.001**
Positive (≥8%)—n (%)	41 (100)	27 (100)	0	0	68 (46)	
Negative (<8%)—n (%)	0	0	54 (100)	27 (100)	81 (54)	
Mean (%)	46	34	3	3	18.5	
10q loss status—n (%)						**<.001**
Positive (including *MGMT*)	41 (100)	0	54 (100)	0	95 (64)	
Negative	0	27 (100)	0	27 (100)	54 (36)	

Note: Significant values are indicated in bold.

Pyrosequencing of *MGMT* PCR products was carried out using PyroMark Q24 Gold Reagents (Qiagen). Finally, the results were interpreted using Pyromark Q24 (Qiagen) software. Representative positive and negative pyrographs are shown in Figure S1. The final methylation percentage was defined as the mean methylation percentage of the five CpG sites. The clinical cutoff for methylation/non-methylation was set at 8%, an optimal risk cutoff first determined by a retrospective study in 2012[22] and subsequently confirmed in a prospective study in 2016.[23]

2.5 | ArrayCGH

This technique was performed on the same extract of DNA used for pyrosequencing exploration; a minimum of 300 ng (37.5 ng/uL) was required. Labeling (Genomic DNA ULS Labeling Kit Agilent), purification, and hybridization of the tumor DNA samples were carried out according to the manufacturer's protocols (Oligonucleotide Array-Based CGH for Genomic analysis, Agilent). The samples were hybridized with the SurePrint G3 Human CGH Microarray Kit 4 × 180 K. The slides (Hybridization Gasket Slide Kit 4-pack microarrays Agilent) were analyzed by Agilent SureScan Dx Microarray Scanner Bundle scanner and the TIFF images were obtained using Agilent Scan Control software. Raw data were generated using Feature Extraction software and analyzed by Agilent Cytogenomics software. The main aberration filter was set to call "copy number variation" when at least five consecutive probes deviated from an absolute $\log^2$ ratio value of 0.25.[24] All profiles were evaluated by qualified molecular biologists. A representative

CGHarray profile with hetorozygous 10q loss is shown in Figure S2.

2.6 | Statistical analyses

Patients were classified into four groups according to their *MGMT* methylation and 10q26.3 loss status. Comparison of patient characteristics by groups was conducted by chi-square test for qualitative variables and Kruskal-Wallis test for quantitative variables. OS and PFS were estimated by the Kaplan-Meier using the log-rank test method and were described using median or rate at specific time points along with their 95% confidence interval (CI). For OS, patients known to be alive were censored at the date of their last follow-up. For PFS, living patients without progression were censored at the date of their last follow-up. Follow-up was calculated by a reverse Kaplan-Meier estimation. Statistical analyses were performed using GraphPadPrism (v6.01) and IBM SPSS Statistics 21 software.

3 | RESULTS

3.1 | Patient and tumor characteristics

All in all, 149 GBM specimens were included. Among them, 68 (46%) were *MGMT* hypermethylated and 81 (54%) were *MGMT* unmethylated, 95 (64%) had 10q26.3 loss, and 54 (36%) had no 10q26.3 loss.

Forty-one tumors (28%) presented dual inactivation of *MGMT* (Group 1: M*GMT* hypermethylated and 10q26.3 loss), 27 tumors (18%) were *MGMT* hypermethylated without 10q26 loss (Group 2), 54 tumors (36%) were *MGMT* unmethylated with 10q26.3loss (Group 3), and 27 tumors (18%) were *MGMT* unmethylated without 10q26.3 loss (Group 4). This distribution is summarized in a graphical representation (Figure 1).

The groups were well balanced with no statistical differences between age, gender, or histobiological data (Table 1).

3.2 | Treatment delivery

At time of diagnosis, the Stupp's protocol was initiated for all patients starting with surgical intervention. Complete surgery, defined as the absence of visible contrast enhancement on post-surgery MRI, was possible for only 62 patients (41.6%) (Table 2). One-hundred and eight patients (72.5%) received 75 mg/m^2/d TMZ concomitant with radiotherapy delivered at a dose of 60 Gy, distributed in 30 fractions of 1.8-2 Gy per day, 5 days per week, over a period of 6 weeks. Median time between surgery and radiochemotherapy was 47 days. Among the 108 patients, 87 (80.6%) received adjuvant TMZ at 150-200 mg/m^2/d according to the Stupp's protocol. Treatment delivery did not differ between groups except by the number of cycles of adjuvant therapy administrated with more cycles received by patients with hypermethylated and 10q26.3 loss tumor (Group 1, $P < .001$) (Table 2). Sixty-eight (46%) patients presented tumor progression with no statistical difference between groups (Table S1). Regarding second line treatment, repeat surgery was more frequently performed in patients with dual inactivation of *MGMT* (31%) ($P = .04$).

3.3 | Overall survival and progression-free survival

After median follow-up of 18.2 months, 118 patients (79.2%) out of 149 had experienced tumor recurrence and 105 (70.5%) had died. Median OS and median PFS for the whole cohort were 10.2 and 6.4 months, respectively (Figure S3). As expected and as previously described (Hegi et al[8]), patients with *MGMT* hypermethylated tumors had significantly longer OS and PFS than patients with *MGMT* unmethylated tumors ($P < .001$ and $P = .0054$, respectively) (Figure S4A,B). No significant OS/PFS difference was observed between patients with or without 10q26.3 LOH tumors (Figure S4C,D).

All in all, *MGMT* promoter methylation and 10q26.3 loss status identified four groups of different prognosis. Patients

FIGURE 1 A graphical representation of overlap or lack thereof of the four prognostic groups

At diagnosis	Group1 (N = 41)	Group2 (N = 27)	Group3 (N = 54)	Group4 (N = 27)	Total (N = 149)	P
Surgery—n (%)	41 (100)	27 (100)	54 (100)	27 (100)	149 (100)	
Biopsy	16 (39)	16 (59)	22 (41)	13 (48)	67 (45)	.35
Type of surgery	25 (61)	11 (41)	32 (59)	14 (52)	82 (55)	
Complete	20 (80)	7 (64)	26 (81)	9 (64)	62 (76)	.76
Partial	5 (20)	3 (27)	6 (19)	4 (29)	18 (22)	
Unknown	0	1 (9)	0	1 (7)	2 (2)	
Concomitant RT + TMZ n (%)	31 (76)	17 (63)	38 (70)	22 (82)	108 (73)	.45
Adjuvant TMZ—n (%)	27 (66)	15 (56)	30 (56)	15 (56)	87 (58)	.73
TMZ cycles						
≥6 cycles	19 (46)	9 (33)	6 (11)	5 (19)	39 (26)	**<.001**
Median [min-max]	8.5 [1-24]	6 [1-20]	3 [1-13]	4.5 [1-14]	5 [1-24]	**.004**
Supportive care alone—n (%)	6 (15)	6 (22)	10 (19)	3 (11)	25 (17)	.69
TMZ alone—n (%)	4 (10)	3 (11)	6 (11)	1 (4)	14 (10)	.5
RT alone—n (%)	0	1 (4)	0	1 (4)	2 (1)	.31

TABLE 2 Therapeutic management of patients at diagnosis

Note: The median is displayed. Significant values are indicated in bold.

Abbreviations: RT, radiotherapy; TMZ, Temozolomid.

with dual *MGMT* inactivation (Group 1, n = 41) presented the longest OS and PFS with median OS of 21.5 months (*P* = .002) and median PFS of 7.2 months (*P* = .03), with 45% of survivors at 2 years compared to Group 2 (24%), Group 3 (0%), and Group 4 (5%) (*P* < .001) (Table 3, Figure 2A,B). Similarly, Group 1 comprised 31% of patients free of progression after 18 months, compared to Group 2 (25%), Group 3 (3%), and Group 4 (6%). Of particular interest, all long-term survivor patients (n = 6, 14.6%) with OS ≥ 30 months belonged to Group 1. No patient in the other groups reached this OS. These results remained the same when *IDH* mutated tumors, for which the predictive influence of *MGMT* methylation does not apply, were excluded (n = 142) (Figure S5).

It is worth noting that OS and PFS were similar during the first 8 months of follow-up, whatever the molecular profile. In patients with hypermethylation of *MGMT* promoter (n = 68), OS tended to be longer in patients with 10q26.3 loss tumors (Group 1) compared to patients without (Group 2; *P* = .12) (Figure 3A, Figure S6). From 8-month follow-up, significantly different OS was observed between these two Groups (*P* = .009; Figure 3B). The hazard ratio of Group 1 with dual inactivation of *MGMT* compared to Group 2 with methylation of *MGMT* alone was 0.33 (95% CI [0.063-0.604]), which corresponded to a 67% decrease in risk of death. While comparing cases in Group 1 and Group 2, who

completed at least six cycles of adjuvant TMZ, OS tended to be statistically different at 8-month follow-up (*P* = .06) but not at diagnosis (*P* = .24, Figure S7). However, the number of patient was too low to draw meaningful conclusions (n = 19 and n = 9 respectively).

3.4 | Univariate and multivariate analysis

Finally, we conducted a uni- and multivariate analysis of well-known markers of interest in GBM in our cohort. Age at diagnosis, the WHO performance status, the extent of surgical resection and dual inactivation of *MGMT* were independent prognostic factors of GBM as they were significantly associated with OS in uni-and multivariate analysis (Table 4). Age at diagnosis, the WHO performance status, extent of surgical resection and dual inactivation of *MGMT* were also significantly associated with PFS in univariate and remained in multivariate analysis.

4 | DISCUSSION

In our study, we investigated OS and PFS in GBM according to *MGMT* promoter methylation profile and chromosome 10q

TABLE 3 OS and PFS according to the *MGMT* gene promoter methylation and 10q chromosome status in the total study population

Features	Group1 (N = 41)	Group2 (N = 27)	Group3 (N = 54)	Group4 (N = 27)
Median follow up (mo)	16.9	18.2	20.5	24.6
Number of deaths—n (%)	24 (59)	18 (67)	41 (76)	22 (82)
Survival *MGMT* (median—month)	15.1		8.9	
Survival (median—month)	21.5	12	8.1	9.5
Overall survival rate (%)				
6 mo	71	67	61	74
12 mo	60	46	36	39
18 mo	57	33	9	10
24 mo	45	24	0	5
Number of patients with progression—n (%)	32 (78)	16 (59)	46 (85)	24 (88)
Progression-free survival *MGMT* (median—month)	6.2		6.4	
Progression-free survival (median—month)	7.2	5.4	6	6.9
Progression-free survival rate (%)				
6 mo	59	48	49	59
12 mo	38	32	10	17
18 mo	31	25	3	6
24 mo	19	25	3	0

Note: Group1: M*GMT* hypermethylated and 10q26.3 loss. Group2: *MGMT* hypermethylated without 10q26 loss. Group3: *MGMT* unmethylated with 10q26.3 loss and Group4: *MGMT* unmethylated without 10q26.3 loss.
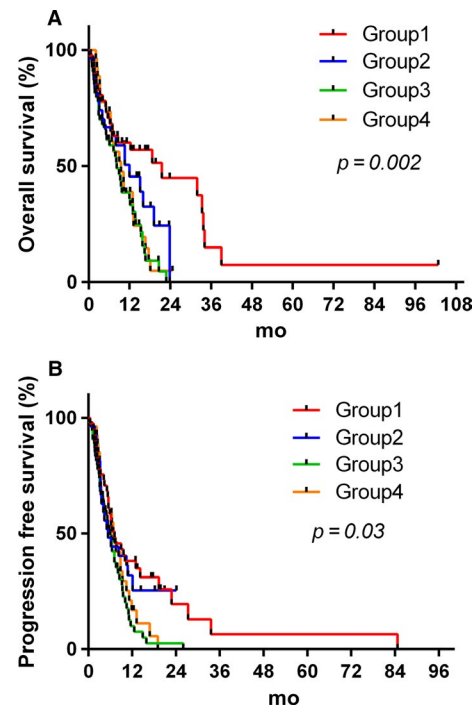
FIGURE 2 Kaplan-Meier curves representing OS (A) and PFS (B) according to *MGMT* gene promoter methylation and chromosome 10q status. Group1: *MGMT* hypermethylated and 10q26.3 loss. Group2: *MGMT* hypermethylated without 10q26 loss. Group3: *MGMT* unmethylated with 10q26.3 loss and Group4: *MGMT* unmethylated without 10q26.3 loss

status and showed that the combination chromosome 10q26 loss with hypermethylation of the *MGMT* promoter in patients with GBM is an interesting prognostic tool associated with longer OS ($P = .002$) and PFS ($P = .03$). Knowledge of this dual inactivation of *MGMT* can enable selection of long-term survivor patients (OS ≥ 30 months).

Our population was representative of classic GBM population and no major selection bias was noted. The mean age at GBM diagnosis was 64 years with an M/F ratio of 1.4 in agreement with epidemiological studies.[1,2,25] Our cohort consisted of 95% primary GBMs and 5% secondary GBMs,

which was consistent with the literature.[26] Among the latter *IDH1* R132H was the most frequent mutation (86%). Hypermethylation of *MGMT* promoter was present in 46% of GBMs and chromosome 10q26.3 loss in 64%. Taken together, these observations corroborated the literature.[27,28]

All patients initiated the Stupp protocol by undergoing surgical procedures. Among them, 73% received concomitant radiochemotherapy after surgical procedure and 58% received adjuvant TMZ. Twenty-six percent received the complete standard treatment (surgery, concomitant radiochemotherapy followed by at least six cycles of adjuvant TMZ). Patients who were not able to receive radiochemotherapy (27%) could instead receive either TMZ alone (9%), or radiotherapy alone (1%) or supportive cares alone (17%). These results were similar to the 2005 Stupp et al study in which only 85% of patients had received radiochemotherapy post-surgery and only 36.6% had received the complete standard treatment.[3] As in the literature, we reported age at diagnosis, WHO performance status and extent of surgical resection as independent prognostic factors.[8,29,30] We did not find any prognostic impact of chromosome 10q loss by itself in our cohort. Data in the literature are conflicting with chromosome 10q loss, sometimes described as a poor prognostic factor[12-15] and sometimes as a non-impact.[16-20]

Median OS of the cohort was short, 10.2 months, compared to the Stupp et al standard 14.6 months. One explanation

could come from the higher number of patients with only biopsy instead of complete resection, 45% compared to 16% in the Stupp et al study. As biopsy resection is known to be a negative prognosis marker compared to complete surgery,[31,32] it is possible that the high percentage of biopsy explains the relatively low median OS of our study. We have no
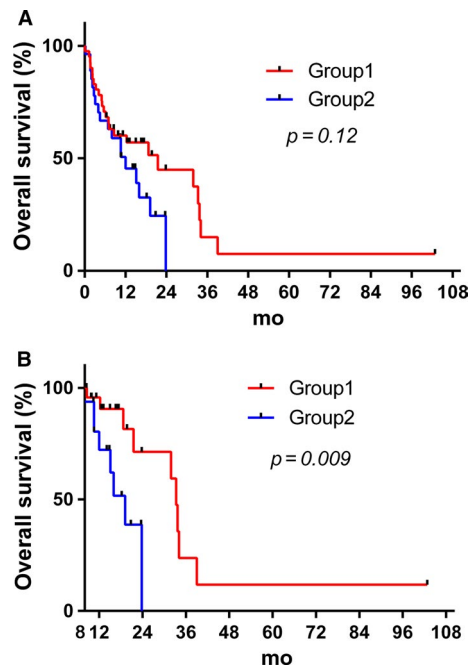

FIGURE 3 Kaplan-Meier curves representing OS according to chromosome 10q status in patients with GBM with hypermethylation of the MGMT gene promoter at diagnosis (A) and at 8-mo follow-up (B). Group1: MGMT hypermethylated and 10q26.3 loss. Group2: MGMT hypermethylated without 10q26 loss

explanation for this high rate of biopsy but it did not cause major bias as the number of biopsies was equally distributed between groups ($P = .76$). Another explanation for the low OS could come from the median delay between surgery and radiotherapy, which was slightly longer than recommended, 47 instead of 42 days (Referential "Association des neuro-oncologues d'expression francaise" 2018). However, the influence of this delay on survival is controversial. An overly lengthy delay would be deleterious, or without influence and even beneficial, depending on studies.[33-37]

When focusing on molecular aspects, patients with GBM with dual mechanisms of _MGMT_ inactivation had longer OS ($P = .002$) and PFS ($P = .03$). In the hypermethylated group (Group 1 + 2; n = 68), patients with loss of chromosome 10q had longer OS from 8-month follow-up than patients without 10q loss ($P = .009$). These results were consistent with the Hegi et al study,[8] in which it was also observed that OS did not differ according to _MGMT_ promoter methylation status during the first 9 months of follow-up. As a result, even though the MGMT promoter methylation is significantly correlated with TMZ response, during the first months of therapeutic management it does not provide reliable prognostic information, whatever the chr10q status of the GBM patients. In GBM studies such as ours, patients can be included at an advanced stage of disease or have altered general state of health, which means that their immediate survival may no longer depend on underlying molecular mechanisms, for example, _MGMT_ methylation status, but rather on other prognostic factors such as age, WHO performance status, co-morbidities or surgical management. Bady et al also investigated the interaction between 10q deletion and _MGMT_ methylation and found

TABLE 4 Uni-and multivariate analyses with Cox proportional-hazards model in the total study population (n = 149) according to OS and PFS

	Univariate analysis			Multivariate analysis		
	_P_	Hazard ratio	CI 95%	_P_	Hazard ratio	CI 95%
Overall Survival						
Gender	.309	0.816	0.552-1.207			
Age at diagnosis	**<.001**	1.037	1.019-1.056	**.006**	1.025	1.007-1.044
WHO	**<.001**	2.078	1.600-2.699	**<.001**	1.974	1.489-2.617
Complete surgery	**<.001**	3.028	1.966-4.665	**<.001**	2.3	1.469-3.601
Methylation _MGMT_ + 10q loss	**.001**	2.306	1.386-3.834	**.001**	2.411	1.433-4.054
IDH	.319	1.795	0.569-5.668			
Progression-Free Survival						
Gender	.593	0.905	0.629-1.303			
Age at diagnosis	**.001**	1.026	1.011-1.042	**.045**	1.016	1.000-1.032
WHO	**<.001**	1.813	1.414-2.325	**<.001**	1.772	1.348-2.329
Complete surgery	**<.001**	2.139	1.445-3.166	**.01**	1.695	1.132-2.537
Methylation _MGMT_ + 10q loss	**.023**	1.639	1.070-2.512	**.022**	1.67	1.078-2.588
IDH	.361	1.52	0.619-3.736			

Abbreviation: CI, confidence interval, Significant values are indicated in bold.

no significant association ($P = .196$) in a TCGA-Glioma-II/III data set.[38] Another team investigated the association between *MGMT* mRNA expression and chr10 copy number and showed a lack of significant differences between cases with chromosome 10 monosomy, *MGMT* locus deletion or normal copy number.[39] However, they did not correlate their results with survival data, and the techniques used for *MGMT* methylation and copy number determination were less sensitive. Finally, another study found no influence of 10q LOH over OS independently of *MGMT* methylation status, but it was performed on a cohort of mix GBM and low grade gliomas.[40]

We observed no PFS differences ($P = .79$) between group 1 and group 2, probably due to the fact that the majority of patients had tumor progression within the first 8 months of follow-up. This lack of association can also be explained by the inherent difficulties of determination of true tumor progression, distinguished from pseudoprogression and radionecrosis.[41] Besides, pseudoprogression is more likely to occur in patients with methylation of the *MGMT* promoter.[42,43]

It is of interest to note that patients with dual *MGMT* inactivation received a higher number of adjuvant TMZ cycles ($P < .001$) during therapeutic management at diagnosis and during revision surgery at tumor progression ($P = .04$). A more intensive treatment might also explain why they lived longer. However, according to our hypothesis, this difference of therapeutic management could in fact be the reflection of the better prognosis of patients from group 1. Indeed, dual inactivation of *MGMT* may increase sensitivity of GBM patients to TMZ treatment and could, therefore, result in a greater number of adjuvant TMZ cures. Due to their maintained general health condition, these patients would then benefit from more frequent revision surgery on tumor progression. Of the 16 patients in this group who relapsed, five (31%) underwent a new surgical procedure for tumor progression compared to only four (15%) out of the 27 patients in the unmethylated group with 10q loss (Group 3) and none in the other two groups. After detailed study of these five patients, it appeared that they all had OS ≥ 30 months. In addition, we observed that among the 41 patients with dual inactivation of *MGMT*, six (15%) survived ≥ 30 months, whereas no patient in the other groups in the study reached this survival time.

One of our study limitations was the lack of statistical power in the hypermethylated group, which allowed us to highlight the interest of 10q loss not from diagnosis but only from 8-month follow-up. This lack of power could be explained by the small size of our cohort (149 with only 27 patients in each group with no 10q loss), linked to technical limitations. Many samples were not eligible for the study due to inconclusive onco-biological results. Among the 259 patients eligible for the study, 110 (42.5%) were excluded: 93 had non-contributory CGHarray analyses, nine had non-contributory pyrosequencing analyses and eight had non-contributory analyses for both techniques. Indeed, quality and, most of all, quantity of tumor DNA extracted from fixed embedded paraffin tissue was frequently insufficient for CGH exploration. However, the use of CGH to study 10q loss is a key feature of our study as most other trials on this subject used LOH analysis by microsatellite markers.[13,14,16,17,19] Despite the need for a large amount of tumor DNA, array CGH in routine practice offers significant advantages over LOH as it is a genome-wide screening technique that can detect deletion of chromosome 10q along with gain of chromosome 7 in GBM and it can also be useful to diagnose oligodendroglioma by detecting 1p19q co-deletion at high resolution. Another limitation of our study is the lack of validation cohort, which would have ascertained our results. Other studies on larger cohorts have previously been conducted, such as The Cancer Genome Atlas (TCGA) or the Chinese Glioma Genome Atlas (CGGA), and the methylation of *MGMT* promoter and the copy number status were part of the data they gathered. However, they did not address the specific question of correlation between 10q loss and *MGMT* methylation, as we did. While looking at TCGA dataset, the number of patients without 10q loss was too low to perform the same analysis, and to draw reliable conclusions. Maybe, they did not use the same technique for copy number determination as ours.

To conclude, the 10q loss associated with hypermethylation of *MGMT* could be identified as a theranostic molecular signature of GBM, enabling selection of patients for whom TMZ was most likely to be beneficial. Given the increasingly systematic nature of the study of chromosome 10q status in integrated histopathological and molecular diagnosis of the WHO 2016 classification, combined with highly recommended study of *MGMT* methylation status, this signature could easily be incorporated into GBM biological and clinical routine. Finally, further prospective study that would include adequately treated patients only (patients who have completed Stupp protocol with at least six cycles of adjuvant TMZ) would provide even more insight on the true prognostic benefit of dual inactivation of *MGMT*.

CONFLICT OF INTEREST
The authors have no conflict of interest related to this work.

AUTHOR CONTRIBUTIONS
All cited authors have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. They have been involved in drafting

the manuscript or revising it critically for important intellectual content; They all have given final approval of the version to be published. They have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REFERENCES

1. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS Statistical Report: primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. *Neuro-Oncol*. 2015;17(suppl 4):iv1-iv62.

2. Reni M, Mazza E, Zanon S, Gatta G, Vecht CJ. Central nervous system gliomas. *Crit Rev Oncol Hematol*. 2017;113:213-234.

3. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987-996.

4. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459-466.

5. Lukas RV, Wainwright DA, Ladomersky E, Sachdev S, Sonabend AM, Stupp R. Newly diagnosed glioblastoma: a review on clinical management. *Oncol Williston Park N*. 2019;33(3):91-100.

6. Bell EH, Zhang P, Fisher BJ, et al. Association of MGMT promoter methylation status with survival outcomes in patients with high-risk glioma treated with radiotherapy and temozolomide: an analysis from the NRG oncology/RTOG 0424 Trial. *JAMA Oncol*. 2018;4(10):1405-1409.

7. Brennan CW, Verhaak RGW, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013;155(2):462-477.

8. Hegi ME, Diserens A-C, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352(10):997-1003.

9. Binabaj MM, Bahrami A, ShahidSales S, et al. The prognostic value of MGMT promoter methylation in glioblastoma: a meta-analysis of clinical trials. *J Cell Physiol*. 2018;233(1):378-386.

10. Silber JR, Bobola MS, Blank A, Chamberlain MC. O(6)-methylguanine-DNA methyltransferase in glioma therapy: promise and problems. *Biochim Biophys Acta*. 2012;1826(1):71-82.

11. Rasheed BK, McLendon RE, Friedman HS, et al. Chromosome 10 deletion mapping in human gliomas: a common deletion region in 10q25. *Oncogene*. 1995;10(11):2243-2246.

12. Balesaria S, Brock C, Bower M, et al. Loss of chromosome 10 is an independent prognostic factor in high-grade gliomas. *Br J Cancer*. 1999;81(8):1371-1377.

13. Ohgaki H, Dessen P, Jourde B, et al. Genetic pathways to glioblastoma: a population-based study. *Cancer Res*. 2004;64(19):6892-6899.

14. Schmidt MC, Antweiler S, Urban N, et al. Impact of genotype and morphology on the prognosis of glioblastoma. *J Neuropathol Exp Neurol*. 2002;61(4):321-328.

15. Jesionek-Kupnicka D, Szybka M, Potemski P, et al. Association of loss of heterozygosity with shorter survival in primary glioblastoma patients. *Pol J Pathol Off J Pol Soc Pathol*. 2013;64(4):268-275.

16. Weller M, Felsberg J, Hartmann C, et al. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. *J Clin Oncol Off J Am Soc Clin Oncol*. 2009;27(34):5743-5750.

17. Houillier C, Lejeune J, Benouaich-Amiel A, et al. Prognostic impact of molecular markers in a series of 220 primary glioblastomas. *Cancer*. 2006;106(10):2218-2223.

18. Wemmert S, Ketter R, Rahnenfuhrer J, et al. Patients with high-grade gliomas harboring deletions of chromosomes 9p and 10q benefit from temozolomide treatment. *Neoplasia N Y N*. 2005;7(10):883-893.

19. Batchelor TT, Betensky RA, Esposito JM, et al. Age-dependent prognostic effects of genetic alterations in glioblastoma. *Clin Cancer Res*. 2004;10(1):228-233.

20. Felsberg J, Rapp M, Loeser S, et al. Prognostic significance of molecular markers and extent of resection in primary glioblastoma patients. *Clin Cancer Res*. 2009;15(21):6683-6693.

21. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol Off J Am Soc Clin Oncol*. 2010;28(11):1963-1972.

22. Quillien V, Lavenu A, Karayan-Tapon L, et al. Comparative assessment of 5 methods (methylation-specific polymerase chain reaction, MethyLight, pyrosequencing, methylation-sensitive high-resolution melting, and immunohistochemistry) to analyze O6-methylguanine-DNA-methyltranferase in a series of 100 glioblastoma patients. *Cancer*. 2012;118(17):4201-4211.

23. Quillien V, Lavenu A, Ducray F, et al. Validation of the high-performance of pyrosequencing for clinical MGMT testing on a cohort of glioblastoma patients from a prospective dedicated multicentric trial. *Oncotarget*. 2016;7(38):61916-61929.

24. Tzetis M, Kitsiou-Tzeli S, Frysira H, Xaidara A, Kanavakis E. The clinical utility of molecular karyotyping using high-resolution array-comparative genomic hybridization. *Expert Rev Mol Diagn*. 2012;12(5):449-457.

25. Baldi I, Huchet A, Bauchet L, Loiseau H. Epidemiology of glioblastoma. *Neurochirurgie*. 2010;56(6):433-440.

26. Louis DN, Perry A, Reifenberger G, et al. The 2016 world health organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol (Berl)*. 2016;131(6):803-820.

27. Hartmann C, Meyer J, Balss J, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol (Berl)*. 2009;118(4):469-474.

28. Crespo I, Vital AL, Gonzalez-Tablas M, et al. Molecular and genomic alterations in glioblastoma multiforme. *Am J Pathol*. 2015;185(7):1820-1833.

29. Mirimanoff R-O, Gorlia T, Mason W, et al. Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. *J Clin Oncol Off J Am Soc Clin Oncol*. 2006;24(16):2563-2569.

30. Aquilanti E, Miller J, Santagata S, Cahill DP, Brastianos PK. Updates in prognostic markers for gliomas. *Neuro-Oncol*. 2018;20(suppl_7):vii17-vii26.

31. Grabowski MM, Recinos PF, Nowacki AS, et al. Residual tumor volume versus extent of resection: predictors of survival after surgery for glioblastoma. *J Neurosurg*. 2014;121(5):1115-1123.

32. Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. *Neurosurgery*. 2008;62(4):753-766; discussion 264–266.

33. De Barros A, Attal J, Roques M, et al. Impact on survival of early tumor growth between surgery and radiotherapy in patients with de novo glioblastoma. *J Neurooncol*. 2019;142(3):489-497.

34. Irwin C, Hunn M, Purdie G, Hamilton D. Delay in radiotherapy shortens survival in patients with high grade glioma. *J Neurooncol*. 2007;85(3):339-343.

35. Lawrence YR, Blumenthal DT, Matceyevsky D, Kanner AA, Bokstein F, Corn BW. Delayed initiation of radiotherapy for glioblastoma: how important is it to push to the front (or the back) of the line? *J Neurooncol*. 2011;105(1):1-7.

36. Louvel G, Metellus P, Noel G, et al. Delaying standard combined chemoradiotherapy after surgical resection does not impact survival in newly diagnosed glioblastoma patients. *Radiother Oncol J Eur Soc Ther Radiol Oncol. janv*. 2016;118(1):9-15.

37. Seidlitz A, Siepmann T, Löck S, Juratli T, Baumann M, Krause M. Impact of waiting time after surgery and overall time of postoperative radiochemotherapy on treatment outcome in glioblastoma multiforme. *Radiat Oncol Lond Engl*. 2015;10:172.

38. Bady P, Delorenzi M, Hegi ME. Sensitivity analysis of the MGMT-STP27 model and impact of genetic and epigenetic context to predict the MGMT methylation status in gliomas and other tumors. *J Mol Diagn*. 2016;18(3):350-361.

39. Ramalho-Carvalho J, Pires M, Lisboa S, et al. Altered expression of MGMT in high-grade gliomas results from the combined effect of epigenetic and genetic aberrations. *PLoS One*. 2013;8(3):e58206.

40. Fontana L, Tabano S, Bonaparte E, et al. MGMT-methylated alleles are distributed heterogeneously within glioma samples irrespective of IDH status and chromosome 10q deletion. *J Neuropathol Exp Neurol*. 2016;75(8):791-800.

41. Ellingson BM, Chung C, Pope WB, Boxerman JL, Kaufmann TJ. Pseudoprogression, radionecrosis, inflammation or true tumor progression? challenges associated with glioblastoma response assessment in an evolving therapeutic landscape. *J Neurooncol*. 2017;134(3):495-504.

42. Gahramanov S, Varallyay C, Tyson RM, et al. Diagnosis of pseudoprogression using MRI perfusion in patients with glioblastoma multiforme may predict improved survival. *CNS Oncol*. 2014;3(6):389-400.

43. Brandes AA, Franceschi E, Tosoni A, et al. MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26(13):2192-2197.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.