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ORIGINAL RESEARCH

Dissecting the prognostic signature of patients with astrocytoma isocitrate dehydrogenase-mutant grade 4: a large multicenter, retrospective study

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Background: The World Health Organization (WHO) 2021 classification of central nervous system (CNS) tumors classified astrocytoma isocitrate dehydrogenase-mutant (A *IDH*m) with either microvascular proliferation and/or necrosis or homozygous deletion of *CDKN2A/B* as CNS grade 4 (CNS WHO G4), introducing a distinct entity and posing new challenges to physicians for appropriate management and prognostication.

Patients and methods: We retrospectively collected information about patients diagnosed with A *IDH*m CNS WHO G4 at three reference neuro-oncological Italian centers and correlated them with survival.

Results: A total of 133 patients were included. Patients were young (median age 41 years) and most received post-operative treatment including chemo-radiation ($n = 101$) and/or temozolomide maintenance ($n = 112$). With a median follow-up of 51 months, the median overall survival (mOS) was 31.2 months, with a 5-year survival probability of 26%. In the univariate analysis, complete resection (mOS: 40.2 versus 26.3 months, $P = 0.03$), *methyl-guanine methyltransferase* (*MGMT*) promoter methylation (mOS: 40.7 versus 18 months, $P = 0.0136$), and absence of *telomerase reverse transcriptase* (*TERT*) promoter mutation (mOS: 40.7 versus 18 months, $P = 0.0003$) correlated with better prognosis. In the multivariate models, lack of *TERT* promoter mutation [hazard ratio (HR) 0.23, 95% confidence interval (CI) 0.07-0.82, $P = 0.024$] and *MGMT* methylation (HR 0.40, 95% CI 0.20-0.81, $P = 0.01$) remained associated with improved survival.

Conclusions: This is the largest experience in Western countries exploring the prognostic signature of patients with A *IDH*m CNS G4. Our results show that *MGMT* promoter methylation and *TERT* promoter mutation may impact clinical outcomes. This may support physicians in prognostication, clinical management, and design of future studies of this distinct diagnostic entity.

Key words: astrocytoma IDH-mutant WHO G4, IDH mutation, adult-type diffuse gliomas

INTRODUCTION

Diffuse gliomas (DGs) are the most common primary brain tumors in adults, with an incidence of ∼15 000 cases per year in the United States.[1] Since the discovery of isocitrate dehydrogenase (*IDH*) mutations, molecular knowledge of these malignancies has increasingly expanded in recent years.[2,3] The 2021 World Health Organization (WHO) classification of central nervous system (CNS) tumors, integrating both histological and molecular information for diagnosis, describes three distinct categories among DG with different clinical behaviors: glioblastoma *IDH* wt CNS WHO grade 4 (GBM *IDH* wt CNS WHO G4), astrocytoma *IDH*-mutant (A IDHm) CNS WHO G2-4, and oligodendroglioma *IDH*-mutant 1p/19q co-deleted (OD *IDH*m) CNS WHO G2-3.[4] A *IDH*m presenting either microvascular proliferation (MVP) and/or necrosis (N) or homozygous deletion of the *cyclin-dependent kinase inhibitor 2 A/B* (*CDKN2A/B*) genes is now classified as CNS WHO G4, with expected longer survival compared with GBM *IDH* wt CNS WHO G4.[2,5]

IDH mutations are located in codon 132 of the *IDH1* gene and codons 172 or 140 of the *IDH2* gene, with *IDH1* R132H

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being the most common (∼90% of cases).[2-4] The mutant enzyme leads to the production and accumulation of 2-hydroxyglutarate, an oncometabolite with a key role in gliomagenesis that induces a wide range of biological effects, such as impaired cellular differentiation and genome-wide hypermethylation.[2-4] Of note, non-R123H *IDH* mutations were associated with increased production of 2-hydroxyglutarate compared with *IDH1* R132H.[6] This seems to translate into higher methylation patterns and improved survival rates in A *IDH*m CNS WHO G2-3.[7,8] Whether the type of *IDH* mutation may impact the prognosis in A *IDH*m WHO G4 remains unknown.

The *methyl-guanine methyltransferase* (*MGMT*) promoter methylation and *telomerase reverse transcriptase* (*TERT*) promoter mutation represent the key molecular alterations contributing to further refine prognostication of DGs in clinical practice.[9-21] *MGMT* is located on chromosome 10 and repairs DNA adducts from alkylating agents such as temozolomide (TMZ).[9] Methylation in the promoter of this gene is a well-defined biomarker in patients with GBM *IDH* wt CNS WHO G4, with hypermethylation associated with better outcomes and increased sensitivity to TMZ.[9,10] The role of *MGMT* appears less clear in the presence of *IDH* mutation.[11-18] In fact, *MGMT* promoter status may lose its prognostic role in *IDH*-mutated tumors as a result of genome-wide hypermethylation and preservation of chromosome 10.[11-18] *TERT* promoter mutation is a molecular alteration commonly found in GBM *IDH* wt and *IDH*m CNS WHO G2-3 OD, with apparent lower frequency in A *IDH*m CNS WHO G4 (3%-5%).[2,4,5,19,20] For both *IDH* wt and *IDH*m populations, the impact of *TERT* on outcomes remains controversial, with contrasting results reported in the literature.[5,21-23] Prognostic and therapeutic implications of both *MGMT* promoter and *TERT* promoter status in A *IDH*m CNS WHO G4 are still not fully elucidated.

The current prognostication of A *IDH*m CNS WHO G4 is generally inferred from reports largely based on GBM *IDH* wt and A *IDH*m CNS WHO G2-3. Thus, after the introduction of the WHO 2021 classification, larger experiences are needed to deeply understand the clinical behavior of this distinct histomolecular entity. This multicenter and retrospective study including patients with A *IDH*m CNS WHO G4 aims to (1) explore the survival of this subpopulation, (2) investigate the impact of several clinical and molecular characteristics on prognosis, (3) put results into context of current evidence about IDH-mutant gliomas.

MATERIAL AND METHODS

Clinical information

We retrospectively reviewed demographic, clinical, and pathological data of consecutive patients diagnosed with adult-type DGs in three reference neuro-oncological Italian centers (IRCCS Humanitas Clinical and Research Hospital, Milan; IRCCS Istituto Oncologico Veneto, Padua; and IRCCS Istituto delle scienze neurologiche, Bologna) between April 2012 and April 2023, identifying those with a WHO diagnosis of GBM *IDH*m or A *IDH*m G4. Available information was reviewed by a neuropathologist to confirm the diagnosis of A *IDH*m CNS WHO G4 according to the current criteria of the WHO 2021 classification, particularly in those cases classified before 2021. We identified a total of 133 cases presenting with (1) *IDH* mutation, (2) absence of 1p-19q co-deletion, and (3) presence of N and/or MVP. The protocol received local ethics approval (ONC-OSS-30-2023). Variables collected were age, sex, anatomical region involved, Karnofsky Performance Status (KPS) after surgery (70% versus ≤70%), use of corticosteroids for neurological symptoms (≥2 mg of dexamethasone or equivalent or not), extent of resection on post-operative imaging [gross total resection or GTR (100% enhancing disease removal) versus no total resection (NTR)], *MGMT* promoter status (methylated versus unmethylated), *IDH* status (R132H versus non-R132H mutations), *TERT* promoter status (wt versus mutant), and type of post-operative therapies delivered.[24]

Efficacy endpoints

We assessed both progression-free survival [PFS; i.e. time from the diagnosis of A *IDH*m CNS WHO G4 to the first progressive disease or death or last follow-up] and overall survival (OS; i.e. time from the diagnosis of A *IDH*m CNS WHO G4 to death or last follow-up) in the entire population and in patients receiving second-line systemic therapies (time from second-line therapy start to death or last follow-up). Progressive disease was defined according to Radiological Assessment in Neuro-oncology (RANO) criteria and/or following a multidisciplinary tumor board discussion.

Molecular analysis

The *IDH1* R132H mutation was detected by immunohistochemistry using an anti-*IDH1* R132H mouse monoclonal antibody.[25] When a next-generation sequencing panel was available or for cases with negative immunohistochemistry but age <55 years, *IDH1* and 2 mutations (R132 in *IDH1* and R172 in *IDH2*) were tested by DNA sequencing.[25] *TERT* promoter mutations were tested by Sanger DNA sequencing.[25,26] The *MGMT* promoter methylation status was assessed by pyrosequencing (cut-off for methylation, 10%).[25,27] When available, the percentage of *MGMT* promoter methylation by pyrosequencing was collected.

Statistical methods

Data were described as numbers and percentages or as median and range. Differences in categorical variables were tested using the chi-square test and those in continuous data using the *t*-test. The follow-up time was estimated with the inverse Kaplan—Meier method. Survival curves were generated using the Kaplan—Meier method. Differences between groups were evaluated using the log-rank test.

The Cox proportional hazards regression model was used to calculate the hazard ratios (HRs) and their 95% confidence intervals (CIs) in both univariate (UVA) and multivariate (MVA) analyses. After checking for correlation between variables and verification of the assumptions of proportional hazard for the Cox model, the final model was built considering all factors statistically significant at the level of $P = 0.1$ (two sides) in the UVA and which confirmed their effect in the multivariate model at the level of $P = 0.05$ (two sides). All analyses were carried out with SAS software, version 9.4 (SAS Institute, Cary, NC).

RESULTS

Clinical characteristics

A total of 133 patients were included in the study. The median age was 41 years (range 18-85 years). Most patients underwent an NTR ($n = 70/133$), and the frontal lobe was the anatomical region more frequently involved ($n = 69/133$). In total, 68% ($n = 90/133$) of cases were newly diagnosed tumors, while the remaining 31% represented forms that evolved from previous WHO G2-3 tumors. Approximately 70% of patients presented a KPS >70% after surgery, with no need for corticosteroids to control neurological symptoms in half of cases. Post-surgical treatments have been administered to ∼90% of patients, including concomitant chemo-radiation with TMZ (76%) and/or TMZ maintenance (86%). For 62 patients having available data on second-line systemic therapies, the most frequent agents used were chemotherapy (nitrosoureas and TMZ, $n = 27$), anti-vascular endothelial growth factor (receptor) [VEGF(R)] agents (regorafenib and bevacizumab, $n = 22$), and experimental immunotherapy within a clinical trial ($n = 11$).

A high frequency of methylation in the *MGMT* promoter was observed ($n = 89/133$). The *IDH* R132H mutation was present in 85% of cases. Meanwhile, 15 patients carried a noncanonical *IDH1/2* mutation (6 cases with *IDH1* R132C, 5 cases with *IDH2* R172K, 1 case with *IDH1* R132G, 1 case with *IDH1* R132L, 1 case with *IDH1* R132S, and 1 case with *IDH2* R172T). The *TERT* promoter mutational status was available for 54 patients, with most being wt ($n = 41/54$) and 13 having a mutation (6 cases with C124T, 5 cases with C228T, 1 case for C146T, and 1 case for C250T). The main characteristics are reported in Table 1.

Table 1. Patients' main characteristics

Variable	Number (%)
Patients	133
Age at diagnosis (years), median (range)	41 (18-85)
Male/female	80/53
Center	
Milan	44
Padua	64
Bologna	25
Setting	
Newly diagnosed	90 (68)
Evolution from G2-3 gliomas	41 (31)
Missing	2 (1)
Previous radiotherapy and/or chemotherapy for G2-G3 gliomas	
Yes	22 (56)
No	19 (44)
KPS after surgery	
>70%	93 (70)
≤70%	29 (22)
Missing	11 (8)
Steroid use	
Yes	52 (39)
No	67 (50)
Missing	14 (11)
Extent of surgery	
GTR	46 (35)
NTR	70 (53)
Missing	17 (12)
IDH status	
R132H	113 (85)
Non-R132H	15 (11)
Missing	4 (4)
MGMT	
Methylated	89 (67)
Unmethylated	21 (16)
Missing	23 (17)
TERT mutation	
Yes	13 (10)
No	41 (31)
Missing	79 (59)
Post-operative therapy	
CT/RT	101 (76)
RT	12 (9)
CT	6 (4)
None	4 (3)
Missing	10 (8)
TMZ concomitant	
Yes	101 (76)
No	11 (8)
Missing	21 (16)
TMZ maintenance	
Yes	112 (86)
No	2 (2)
Missing	19 (12)
Second-line therapy	
Yes	62 (47)
No	40 (30)
Missing	31 (23)
Type of second-line therapy	
Chemotherapy	27/62 (44)
Anti-VEGF(R)	22/62 (35)
Experimental immunotherapy	11/62 (18)
Others or missing	2/62 (3)

CT, chemotherapy; GTR, gross total resection; IDH, isocitrate dehydrogenase; KPS, Karnofsky status; MGMT, O^6-methylguanine-DNA methyltransferase; NTR, no total resection; RT, radiotherapy; TERT, telomerase reverse transcriptase; TMZ, temozolomide; VEGF(R), vascular endothelial growth factor (receptor).

Survival and exploratory analyses

With a median follow-up of 51 months (range 1.1-128.7 months), a total of 96 patients experienced a PFS event and a total of 76 patients were dead. The median PFS (mPFS) was 16.2 months and the median OS (mOS) was 31.2 months. At 2 and 5 years, the survival probability was 60% and 26%, respectively (Figure 1A).

The prognosis of patients with primary and secondary A *IDH*m CNS WHO G4 did not seem to differ (mPFS: 16.9 versus 13.1 months, $P = 0.20$; mOS: 34.5 versus 26.6 months, $P = 0.76$, respectively; Figure 1B). In the second-line setting, all types of systemic therapy performed similarly (mOS: 7.4 versus 15.4 versus 14 months, $P = 0.13$, respectively; Figure 1B).

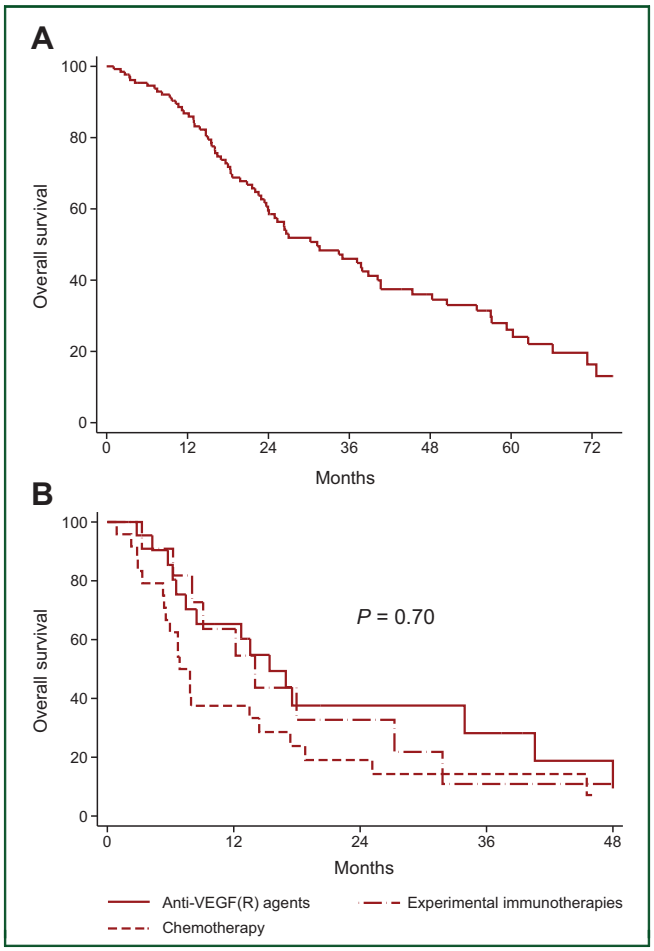

Figure 1. (A) Overall survival in the entire cohort; (B) overall survival according to second-line therapies.
VEGF(R), vascular endothelial growth factor (receptor).

Univariate analysis for PFS and OS

Among clinical variables, a GTR correlated with a favorable outcome (mPFS: 21.8 versus 15.4 months, $P = 0.09$; mOS: 40.2 versus 26.4 months, $P = 0.03$, respectively). By contrast, age at diagnosis (PFS: HR 1.01, 95% CI 0.99-1.02, $P = 0.36$; OS: HR 1.02, 95% CI 1.00-1.04, $P = 0.07$), no need for corticosteroids (mPFS: 20.3 versus 14.0 months, $P = 0.28$; mOS: 38.8 versus 26.6 months, $P = 0.09$ respectively), and a KPS >70% (mPFS: 16.9 versus 12.2 months, $P = 0.24$; mOS: 34.5 versus 24.1 months, $P = 0.14$, respectively) were not associated with a statistically significant improvement in survival.

Among molecular traits, patients with *MGMT* promoter methylation showed a better outcome (mPFS: 23.7 versus 12.0 months, $P = 0.10$; mOS: 40.7 versus 18 months, $P = 0.01$, respectively; Figure 2A). The percentage of pyrosequencing was available for 55 patients. In this case, neither a progressive increasing value (PFS: HR 0.34, 95% CI 0.05-2.18, $P = 0.26$; OS: HR 0.17, 95% CI 0.02-1.65, $P = 0.13$) nor a cut-off of ≥30% (mPFS: 22.0 versus 25.3 months, $P = 0.72$; mOS: 57.1 versus 37.1 months, $P = 0.30$, respectively) was significantly prognostic, with a trend to longer survival for higher methylation in both cases. Patients carrying the

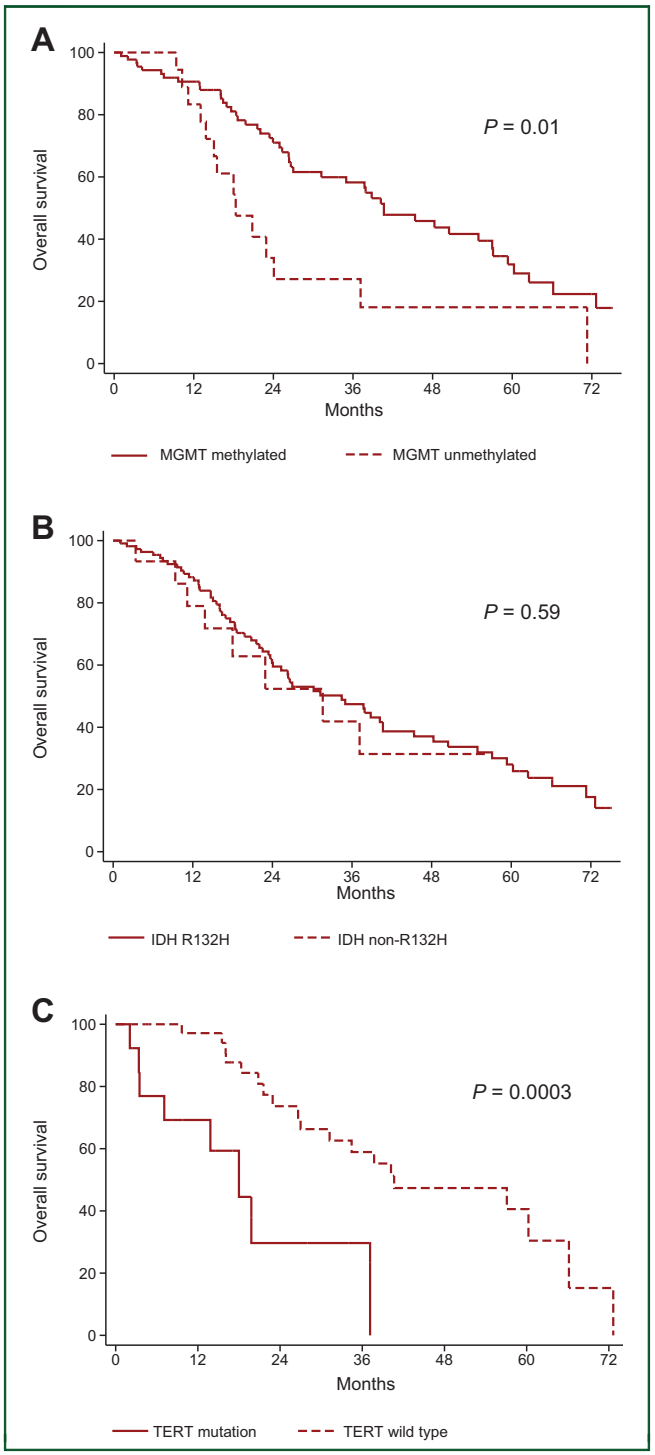

Figure 2. (A) Overall survival for MGMT status; (B) overall survival for the type of IDH mutation; and (C) overall survival for TERT status.
IDH, isocitrate dehydrogenase; MGMT, O^6-methylguanine-DNA methyltransferase; TERT, telomerase reverse transcriptase.

IDH1 R132H mutation and those with non-canonical mutations had similar outcomes (mPFS: 16.9 versus 14.8 months, $P = 0.20$; mOS: 34.5 versus 31.6 months, $P = 0.60$ respectively; Figure 2B). Of note, a significant association between cases with *MGMT* promoter methylation and those with *IDH* R132H mutation was found (chi-square test, $P < 0.0001$). *TERT* promoter mutation demonstrated a

Table 2. Univariate analysis results

Analysis	Median PFS, months	*P* value	Median OS, months	*P* value
KPS after surgery		0.24		0.14
>70%	16.9		34.5	
≤70%	12.2		24.1	
Setting		0.20		0.76
Newly diagnosed	16.9		34.5	
Evolution from G2-3 gliomas	13.1		26.6	
Age		0.36		0.07
Continuous	HR 1.01 (95% CI 0.99-1.02)		HR 1.02 (95% CI 1.00-1.04)	
Steroid use		0.28		0.09
Yes (≥2 mg DXT)	14		26.6	
No	20.3		38.8	
Extent of surgery		0.09		*0.03*
GTR	21.8		40.2	
NTR	15.4		26.4	
IDH status		0.20		0.60
R132H	16.9		34.5	
Non-R132H	14.8		31.6	
MGMT promoter		0.10		*0.01*
Methylated	23.7		40.7	
Unmethylated	12		18	
TERT promoter mutation		*0.01*		*0.0003*
Yes	10.6		18	
No	18.2		40.7	

Statistically significant results (at $P \leq 0.05$) are in italics.
CI, confidence interval; DXT, dexamethasone; GTR, gross total resection; HR, hazard ratio; IDH, isocitrate dehydrogenase; KPS, Karnofsky status; MGMT, O^6-methylguanine-DNA methyltransferase; NTR, no total resection; OS, overall survival; PFS, progression-free survival; TERT, telomerase reverse transcriptase.

negative impact on survival (mPFS: 10.6 versus 18.2 months, $P = 0.01$; mOS: 18 versus 40.7 months, $P = 0.0003$, respectively; Figure 2C). A summary of the UVA is reported in Table 2.

Multivariate analysis for PFS and OS

For OS, *MGMT* promoter methylation, *TERT* promoter status, extent of surgery, age at diagnosis, and need for corticosteroids entered the MVA model and the absence of *TERT* promoter mutation confirmed its favorable impact on survival (HR 0.23, 95% CI 0.07-0.82, $P = 0.02$). The results of the MVA for both PFS and OS are presented in Table 3.

Of note, the MVA model including *TERT* promoter status (model A) limited the analysis to 47 patients. When *TERT* is removed from the MVA, a broader population could be selected (91 patients for OS analysis). In this second MVA model (model B), *MGMT* promoter methylation correlated with a higher OS (HR 0.40, 95% CI 0.20-0.81, $P = 0.01$). The results of MVA model B for OS are presented in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103485.

Survival analysis in the subset of patients affected by primary tumors

To deeply understand the prognostic features of patients with newly diagnosed A *IDH*m CNS WHO G4, a survival analysis limited to this subpopulation was also carried out (90 patients, 68% of cases). In the UVA, KPS >70% after surgery (mOS: 37.7 versus 18 months, $P = 0.003$), age as a continuous variable (HR 1.03, 95% CI 1.01-1.05, $P = 0.003$), use of steroids (mOS: 26.3 versus 50.5 months, $P = 0.035$), and the presence of *TERT* promoter mutation (mOS: 18 versus 57.1 months, $P < 0.001$) significantly impacted OS. However, considering all factors statistically significant at $P < 0.1$, only a few patients may enter a MVA model ($n = 36$), precluding any reliable analysis. Given this, firstly a MVA model (model C) including all variables out of *TERT* promoter status was built, reaching a broader population for analysis and confirming the prognostic significance of age (HR 1.03, 95% CI 1.00-1.06, $P = 0.04$) and *MGMT* promoter methylation (HR 0.42, 95% CI 0.19-0.95, $P = 0.04$). Then, *TERT* promoter status was included with these two variables in another MVA model (model D) and its absence remained the

Table 3. Multivariate analysis results (model A)

Variables	HR for PFS (95% CI)	*P* value	HR for OS (95% CI)	*P* value
MGMT promoter methylation (reference yes)	0.67 (0.27-1.68)	0.39	0.57 (0.17-1.93)	0.36
TERT promoter mutation (reference no)	0.42 (0.17-1.04)	0.06	0.23 (0.07-0.82)	*0.02*
Extent of surgery (reference GTR)	0.49 (0.23-1.01)	0.053	0.53 (0.20-1.42)	0.21
Age (continuous)			1.00 (0.95-1.05)	0.89
Corticosteroid use (reference no)			0.60 (0.22-1.60)	0.31

Statistically significant results (at $P \leq 0.05$) are in italics.
CI, confidence interval; GTR, gross total resection; HR, hazard ratio; MGMT, O^6-methylguanine-DNA methyltransferase; OS, overall survival; PFS, progression-free survival; TERT, telomerase reverse transcriptase.

only factor significantly associated with a better outcome (HR 0.18, 95% CI 0.04-0.82, $P = 0.03$). Supplementary Tables S2 and S3, available at https://doi.org/10.1016/j.esmoop.2024.103485, report the summary of the UVA and MVA for this subgroup of patients, respectively.

DISCUSSION

The WHO 2021 classification of CNS tumors establishes a diagnosis of adult-type DG integrating both histological and molecular features.[4] Different entities have been introduced, including A *IDH*m CNS WHO G4 for which no large amount of information is available so far.[4] A deeper understanding of the biology and clinical behavior of this tumor may help clinicians in prognostication and therapeutic management.

To our knowledge, the present series represents the largest multicenter retrospective experience on patients with A *IDH*m CNS WHO G4 in Western countries ($n = 133$).[13,18-20,28,29] With a long median follow-up period (51 months) and a high number of events (>50% at the time of analysis), providing high reliability to the survival analysis, the median overall survival (mOS) was 31.2 months, consistent with previous suggestions.[2,5,29] Patients were generally young (median age 41 years), as expected in patients diagnosed with an *IDH*m astrocytoma. Consistent with the behavior of high-grade gliomas, most patients presented with newly diagnosed tumors (70% of cases) and received a post-operative treatment with concomitant chemoradiation (76%) and/or TMZ maintenance (86%). In contrast to previous findings from an Asiatic cohort, we did not observe a significant survival difference between secondary and *de novo* tumors (mOS: 26.6 versus 34.5 months, respectively).[26] A potential relationship among genome-wide hypomethylated phenotype, higher grade, and previous medical treatments (radiotherapy and/or chemotherapy) at recurrence was suggested for *IDH*m gliomas.[30,31] In our series, the low number of patients having received medical treatments before their CNS WHO G4 diagnosis ($n = 22/41$) and precluding solid analysis may explain this result. Among clinical factors, the extent of resection only was significantly associated with survival, with a high proportion of patients with NTR (53%). This last observation appears consistent with other series including *IDH* gliomas such as the CATNON trial, in which only 31% of patients received a GTR.[32] Although we did not observe a statistically significant difference among chemotherapy, anti-VEGF(R) agents, and experimental immunotherapy in a subset of patients receiving a second-line systemic therapy, it is worth highlighting a trend to longer survival for those receiving antiangiogenic and immunotherapeutic drugs over alkylating agents (mOS: 14-15 versus 7 months, respectively). This underlines the importance of including recurrent CNS WHO G4 gliomas in a clinical trial whenever possible, as suggested by recent guidelines.[2]

Among molecular variables, patients having *MGMT* promoter methylation had improved survival in both UVA (mOS: 40.7 months) and models B (HR 0.40) and C of primary tumors only (HR 0.42). In the current literature, no definitive conclusion may be drawn on the survival impact of *MGMT* status in patients with A *IDH*m CNS WHO G2-3.[11-18,28] However, our large series described the prognostic significance of *MGMT* promoter methylation for A *IDH*m CNS WHO G4. The frequency of *MGMT* promoter methylation is in line with a recent experience (67% versus 74% of cases), with the lack of promoter methylation noted in a subset of patients potentially explained by the low genome-wide methylation in the case of CNS WHO G4.[18] As seen in a previous report correlating a higher cut-off value of pyrosequencing (≥30%) with favorable outcomes, we observed a not significant but still meaningful trend in survival with this threshold (mOS: 57.1 versus 37.1 months).[15] Patients with different types of *IDH* mutation (R132H versus non-R132H) showed similar survival rates, in contrast to pilot studies reporting improved prognosis for patients having A *IDH*m CNS WHO G2-3 carrying non-R132H mutations.[7,8] However, the low number of patients with noncanonical mutations ($n = 15$) and an association between *MGMT* promoter methylation and R132H mutation could have hidden any difference between these two subpopulations. Given this, *IDH* and *MGMT* promoter status seemed to possess a slightly divergent significance between CNS WHO G2-3 and WHO G4 gliomas. It is conceivable that A *IDH*m CNS WHO G4 presented a different molecular background, with a divergent biological history with less meaningful impact of *IDH* mutation. Patients with *TERT* promoter mutation experienced poor prognosis compared with those with a wild-type sequence in the UVA (mOS: 18 versus 40.7 months) and both MVA models A (HR 0.23) and D including only primary astrocytoma (HR 0.18). To our knowledge, this is the first experience including a large cohort of patients with A *IDH*m CNS WHO G4 to show a prognostic significance of *TERT* status in an MVA model. The most likely explanation is that *TERT* promoter mutation may be a signature of biological aggressiveness, as seen in A *IDH* wt lacking MVP and/or N.[33]

Overall, our experience suggested a prognostic significance of *MGMT* promoter and *TERT* promoter status in patients with A *IDH*m CNS WHO G4. In our view, this may help clinicians to refine prognostication, recognizing patients with a more aggressive disease needing intensive treatment or those with more indolent evolution over time for which other strategies such as various combinations of radiation and chemotherapy may be discussed within appropriate, well-designed clinical trials. Moreover, in light of the recent exciting results of *IDH* inhibitors as treatment for CNS WHO G2 gliomas, a potential role for targeted therapies in patients with higher-grade disease but longer expected survival may represent an intriguing topic to be explored in the future.[34,35]

The WHO 2021 classification simplified the diagnostic process and improved the design of clinical trials. However, A *IDH*m CNS WHO G4 still falls into a '*grey area*' of poor evidence between GBM *IDH* wt and lower-grade *IDH*m gliomas, as also reflected in the most recent guidelines.[2] Large prospective studies specifically addressing this distinct

histomolecular entity should be pursued to improve our knowledge about the disease and to bring high-quality recommendations into clinical practice.

Our study has several limitations. First, this is a retrospective experience with unpredictable bias and missing data. However, this is expected for retrospective studies, and the experience remains relevant as the largest multicenter cohort from Western countries on this topic. Second, all patients were classified as CNS WHO G4 for the presence of MVP and/or N. It remains unknown whether these results may be applied to A *IDH*m without these histological features but presenting *CDKN2A/B* homozygous deletion, now classified as CNS WHO G4 as well.[2] Still, tumors with MVP and/or N are the most frequently encountered in clinical practice, *CDKN2A/B* status is not always tested in these cases and so far we have no strong reason to suggest that the performance of prognostic variables would differ based on how a tumor is classified as CNS WHO G4.[18] Third, other well-established molecular biomarkers of poor prognosis for A *IDH*m, such as methylation profile, *platelet-derived growth factor receptor alpha* amplification, and *phosphoinositide 3-kinase* mutations, were not available.[36,37] Nevertheless, these molecular alterations are usually not easily obtainable in many facilities, and exploring ready-to-use variables such as *IDH* status, *MGMT* promoter, and *TERT* promoter remains of value for clinical practice. Finally, we were not able to retrieve details on the degree of surgical resection in patients having received NTR. This may be expected in retrospective series and future studies should prospectively assess the prognostic value of resection classes and residual volume in this subset of patients as well.[38]

In conclusion, this is the largest multicenter retrospective cohort in Western countries analyzing the prognostic significance of several clinical and molecular variables in patients with A *IDH*m CNS WHO G4. When compared with GBM *IDH* wt, we observed a longer survival time and a similar intensive post-operative chemoradiation approach.[2] We suggest that *MGMT* promoter methylation and *TERT* promoter mutation may affect clinical outcomes, with potential implications for prognostication and clinical management of these patients. These observations, however, warrant further investigation within large, prospective trials.

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DISCLOSURE

The authors have declared no conflicts of interest.

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