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Characteristics of H3 K27M-mutant gliomas in adults

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Abstract

Background. Diffuse H3 K27M-mutant gliomas occur primarily in children but can also be encountered in adults. The aim of this study was to describe the characteristics of H3 K27M-mutant gliomas in adults.

Methods. We analyzed the characteristics of 21 adult H3 K27M-mutant gliomas and compared them with those of 135 adult diffuse gliomas without histone H3 and without isocitrate dehydrogenase (IDH) mutation (IDH/H3 wild type).

Results. The median age at diagnosis in H3 K27M-mutant gliomas was 32 years (range: 18–82 y). All tumors had a midline location (spinal cord $n = 6$, thalamus $n = 5$, brainstem $n = 5$, cerebellum $n = 3$, hypothalamus $n = 1$, and pineal region $n = 1$) and were IDH and *BRAF-V600E* wild type. The identification of an H3 K27M mutation significantly impacted the diagnosis in 3 patients (14%) for whom the histological aspect initially suggested a diffuse low-grade glioma and in 7 patients (33%) for whom pathological analysis hesitated between a diffuse glioma, ganglioglioma, or pilocytic astrocytoma. Compared with IDH/H3 wild-type gliomas, H3 K27M-mutant gliomas were diagnosed at an earlier age (32 vs 64 y, $P < .001$), always had a midline location (21/21 vs 21/130, $P < .001$), less frequently had a methylated *MGMT* promoter (1/21 vs 52/129, $P = .002$), and lacked *EGFR* amplification (0/21 vs 26/128, $P = .02$). The median survival was 19.6 months in H3 K27M-mutant gliomas and 17 months in IDH/H3 wild-type gliomas ($P = .3$).

Conclusion. In adults, as in children, H3 K27M mutations define a distinct subgroup of IDH wild-type gliomas characterized by a constant midline location, low rate of *MGMT* promoter methylation, and poor prognosis.

Key words

glioma | histone | H3 K27M | IDH

Importance of the study

In the revised 2016 World Health Organization classification, "Diffuse midline glioma, H3 K27M-mutant" is recognized as a distinct entity that corresponds to grade IV, even when mitotic figures, microvascular proliferation, and necrosis are not observed. Although these gliomas primarily occur in children, they can also be encountered in adults. In the present study we show that, in adults, as in children, H3 K27M mutations define a distinct subgroup of adult IDH wild-type gliomas characterized by a constant midline location, low rate of *MGMT* promoter methylation, and poor prognosis. The potentially misleading histological presentation of these tumors supports the assessment of H3 K27M mutations in adult IDH wild-type midline gliomas to ensure appropriate diagnosis and grading.

Somatic gain-of-function mutations in genes encoding the histone H3 variants H3.3 and H3.1 occur in the majority of midline diffuse pediatric gliomas and in a subset of pediatric supratentorial high-grade gliomas.[1,2] Lysine to methionine substitution at codon 27 in the *H3F3A* or in the *HIST1H3B/C* genes (H3 K27M mutations) is associated with aggressive diffuse midline gliomas.[3] In contrast, glycine to arginine (or valine) substitution at codon 34 in the *H3F3A* gene is associated with hemispheric glioblastomas.[3] These mutations are mutually exclusive and are also exclusive with *IDH1* and *IDH2* mutations, which are found in most adult low-grade and anaplastic diffuse gliomas.[3] The characteristics of gliomas with H3 K27M mutations have been described in detail in the pediatric population.[3–7] In the revised 2016 World Health Organization (WHO) classification, "Diffuse midline glioma, H3 K27M-mutant" is recognized as a distinct entity that corresponds to grade IV, even when mitotic figures, microvascular proliferation, and necrosis are not observed.[8] Although H3 K27M-mutant gliomas occur primarily in children, they can also be encountered in adults.[7,9–13] However, the characteristics of adult H3 K27M-mutant gliomas have yet to be specifically described. The aim of the present study was to report the characteristics of adult diffuse gliomas with H3 K27M mutations and to compare them with those of adult diffuse gliomas without histone H3 or isocitrate dehydrogenase (IDH) mutation (IDH/H3 wild type).

Patients and Methods

We retrospectively identified all adult patients with gliomas with H3 K27M mutations in our database and reviewed their clinical, radiological, histological, and molecular characteristics. A series of 135 adult patients with diffuse gliomas without *IDH1/2* mutation and without *H3F3A/HIST1H3B* mutations (IDH/H3 wild-type gliomas) was used as a comparative group. *IDH1/2* and *H3F3A/HIST1H3B* mutation status were determined in all cases and *MGMT* promoter methylation and *EGFR* amplification status in most cases (Table 1 and Table 2). *TERT* promoter and *BRAF V600E* mutations were only assessed in H3 K27M-mutant gliomas. DNA was extracted from formalin-fixed paraffin-embedded samples using a standard protocol (Qiagen, QIAmp DNA Mini Kit). After PCR amplification, the mutations of codon 132 of *IDH1*, codon 172 of *IDH2*, codons 27 and 34 of *H3F3A*, codon 28 of *HIST1H3B*, codon 600 of *BRAF*, and mutational hotspots C228 and C250 of the *TERT* promoter were investigated by single-nucleotide primer extension with the ABI PRISM SNaPshot Multiplex kit (Applied Biosystems). PCR and extension primers are listed in Supplementary Table 1.[12,14] *MGMT* promoter methylation was assessed by pyrosequencing. Pyrosequencing was performed with the PyroMark Q96 MGMT kit (Qiagen) on a PSQTM96 MA system (Biotage), as described previously.[15] *EGFR* gene amplification was detected using SYBR Green real-time quantitative PCR analysis (Absolute SYBR Green Rox Mix; Abgene) with the following primers EGFR-F: GTGCAGATCGCAAAGGTAATCAG and EGFR-R: GCAGACCGCATGTGAGGAT; hydrolysis probe: FAM CCCCTCCCCCGTATCTC.[16] Categorical comparisons were performed using Fisher's exact test, and a *t*-test was used for quantitative variables. The survival time was measured from the date of diagnosis to the date of last follow-up or death. Survival was estimated using the Kaplan–Meier method, and differences between curves were assessed using the log-rank test. This study has been approved by our institutional review board.

Results

A total of 21 adult patients with H3 K27M-mutant diffuse gliomas diagnosed between 2011 and 2016 were identified. Their characteristics are summarized in Tables 1 and 2. Twenty patients had an *H3F3A* K27M mutation and one patient an *HIST1H3B* K27M mutation. The median age at diagnosis was 32 years (range: 18–82 y); 7 patients were >40 years of age. The clinical presentation consisted of a variable association of intracranial hypertension (n = 6, 29%), ataxia (n = 5, 24%), cranial nerve injury (n = 4, 19%), and progressive sensorimotor deficits (n = 7, 67%). MRI scans were available for radiological review in 18 patients. All tumors had a midline location (Fig. 1). The most frequent locations were the spinal cord (n = 6, 29%), thalamus (n = 5, 24%), and brainstem (midbrain n = 1, pons n = 1, floor of the fourth ventricle n = 1, medulla oblongata n = 2; total n = 5, 24%). Other locations included the cerebellum (n = 3, 14%), pineal region (n = 1, 5%), and hypothalamus (n = 1, 5%). Contrast enhancement was present in 9 cases (50%) with a ringlike pattern in 8 patients. The shape of the tumor borders was sharp in 4 patients (23%) and blurred in 14 patients (77%). One patient presented with an exophytic tumor that was located on the floor of the fourth ventricle (patient 15) and one patient presented with signs of leptomeningeal involvement at diagnosis (patient 4).

Table 1 Characteristics of 21 adult patients with diffuse glioma, H3 K27M-mutant

Patient Number	Type of Mutation	Age (y)	Sex	Location	Initial Histological Diagnosis	Contrast Enhancement	IDH mut.	EGFR amp.	MGMT meth.	BRAF mut.	pTERT mut.	Treatment	Overall Survival (mo)
1	H3.3 K27M	18	F	Medulla obl.	PA or LGG	No	No	No	No	No	No	B, RT	24
2	H3.3 K27M	18	F	Medulla obl.	GG or HGG	No	No	No	No	No	NA	B, RT/TMZ	8+
3	H3.1 K27M	19	F	Spinal cord	GBM	Yes	No	No	No	No	No	S	1.5
4	H3.3 K27M	20	M	Spinal cord	GBM	Yes	No	No	No	No	No	B, RT/TMZ	7
5	H3.3 K27M	21	F	Pineal region	PA or LGG	Yes	No	No	No	No	No	B	3
6	H3.3 K27M	22	F	Thalamus	AII	No	No	No	No	No	No	B, NU	10+
7	H3.3 K27M	22	F	Hypothalamus	OII	No	No	No	No	No	No	B, RT/TMZ	24
8	H3.3 K27M	23	M	Midbrain	GBM	Yes	No	No	No	No	No	B	1.1
9	H3.3 K27M	31	M	Spinal cord	GBM	Yes	No	No	No	No	No	B, RT	1.3
10	H3.3 K27M	31	F	Cerebellum	GBM	No	No	No	No	No	No	B, RT, BVZ	58
11	H3.3 K27M	33	F	Thalamus	AII	No	No	No	No	No	No	B, RT/TMZ	25
12	H3.3 K27M	34	M	Spinal cord	GG or LGG	Yes	No	No	No	No	Yes	B, RT/TMZ	37
13	H3.3 K27M	34	M	Cerebellum	GBM	Yes	No	No	No	No	No	B, NU	0.7
14	H3.3 K27M	36	F	Thalamus	GBM	Yes	No	No	No	No	No	B, RT/TMZ	17+
15	H3.3 K27M	42	M	Floor of 4th ventricule	GG or LGG	No	No	No	No	No	Yes	S, RT/TMZ	12.8
16	H3.3 K27M	43	F	Spinal cord	PA or LGG	NA	No	No	No	No	No	B	6
17	H3.3 K27M	45	F	Thalamus	GBM	No	No	No	No	No	No	B, RT/TMZ	14+
18	H3.3 K27M	48	M	Pons	GBM	No	No	No	No	No	NA	B, RT/TMZ	19.6
19	H3.3 K27M	52	M	Spinal cord	GG or HGG	NA	No	No	No	No	No	S, RT/TMZ	15+
20	H3.3 K27M	68	M	Cerebellum	GBM	NA	No	No	No	No	No	S, RT/TMZ	2+
21	H3.3 K27M	82	F	Thalamus	GBM	Yes	No	No	Yes	No	No	B,TMZ	5.5

CN: cranial nerve palsy, IH: intracranial hypertension, F: female, M: male, NA: not available, GBM: glioblastoma multiforme, AII/III: astrocytoma grade II/grade III, PA: pilocytic astrocytoma, GG: ganglioglioma, HGG: diffuse high-grade glioma, LGG: diffuse low-grade glioma, IDH mut.: isocitrate dehydrogenase mutation, MGMT meth: MGMT promoter methylation, BRAF mut.: BRAF V600E mutation; pTERT mut.; TERT promoter mutation; B: biopsy, S: surgery, RT: radiotherapy, RT/TMZ: radiotherapy with concurrent and adjuvant temozolomide, NU: nitrosourea, BVZ: bevacizumab, and TMZ: temozolomide. Overall survival, "+": alive at last known status.

Table 2 Comparison of the characteristics of adult patients with H3 K27M-mutant gliomas or with *IDH* wild-type gliomas without histone mutation

	H3 K27M-mutant Gliomas	H3/IDH Wild-Type Gliomas	*P*-value
N	21	135	
Median age at diagnosis, y	32	64	.001
Sex ratio, M/F	0.6/1	0.6/1	
Location, *n* (%)			
Hemispheric	0/21 (0)	109/130 (84)	.001
Diencephalic*	7/21 (33)	10/130 (7)	.003
Brainstem	5/21 (24)	3/130 (2)	.001
Cerebellum	3/21 (14)	6/130 (5)	.008
Spinal cord	6/21 (29)	2/130 (1)	.001
WHO 2007 grading, *n* (%)			
Grade II	3/21 (14)	5/135 (<4)	.07
Grade III	0/21 (5)	17/135 (13)	.1
Grade IV	11/21 (57)	108/135 (80)	.001
Difficult	7/21 (33)	5/135 (<4)	.002
Molecular characteristics, *n* (%)			
MGMT meth.	1/21 (5)	52/129 (40)	.001
EGFR amp.	0/21	26/128 (20)	.02
BRAF V600E mut.	0/21	NA	
pTERT mut.	2/19 (10)	NA	
Median overall survival (mo)	19.6	17	.3

Diencephalic: includes the thalamus, hypothalamus, and pineal region; MGMT meth: MGMT promoter methylation, BRAF mut.: BRAF V600E mutation; pTERT mut: TERT promoter mutation; NA: not available.

Perfusion MRI demonstrated signs of neoangiogenesis in 4 out of the 5 patients in whom it was performed. In 2 patients (patients 9 and 16) the initial MRI was interpreted as a myelitis, yet rapid clinical and radiological deterioration led to histological analysis of the spinal cord lesion. In 3 patients, repeated MRI performed before treatment onset demonstrated a rapid increase in the tumor volume over 2 months in 2 patients (patients 9 and 15) and a slower growth over 32 months in 1 patient (patient 1, Fig. 1).

According to the 2007 WHO classification, histopathological analysis was suggestive of a diffuse glioma in 14 patients (67%) (Fig. 2). In these patients the diagnosis was a glioblastoma (n = 11, 52%), low-grade astrocytoma (n = 2, 10%), and low-grade oligodendroglioma (n = 1, 5%). However, in 7 patients (28%), histological analysis was difficult; due to the presence of piloid features and/or ganglionic differentiation (Fig. 2), the histopathological diagnosis hesitated between a diffuse glioma and ganglioglioma in 4 patients (patients 2, 12, 15, and 19) and between a diffuse glioma and pilocytic astrocytoma in 3 patients (patients 1, 5, and 16). Olig2 staining was positive in all tumors, as was tumor protein p53 staining in 11 cases (55%). The median Ki67 labeling was 15% (range: 0 to 50%). All cases were *IDH* wild type and none harbored *EGFR* amplification. Only one case had a methylated *MGMT* promoter. None of the patients had a *BRAF*V600E mutation. No KIAA49–BRAF fusion was identified in the 3 patients with an initial suspicion of pilocytic astrocytoma. A C228T *TERT* promoter mutation was identified in 2 of 19 (10%) cases in which it was studied. After histopathological review and molecular analysis, all cases corresponded to diffuse midline gliomas, H3 K27M-mutant according to the 2016 WHO classification.

Surgery consisted of a biopsy in 16 patients (76%) and partial surgical resection in 5 patients (24%). After surgery, 14 patients (67%) received radiotherapy that was associated with concurrent and adjuvant temozolomide in 12 patients (57%). Seven patients (33%) could not receive radiotherapy due to rapid clinical deterioration and were treated with chemotherapy alone (n = 4, 19%) or received palliative care (n = 3, 14%). Median survival was 19.6 months in the entire series and 25 months in the subgroup of patients who received radiotherapy with or without temozolomide (Fig. 3).

The characteristics of the 135 IDH/H3 wild-type gliomas are summarized in Table 2. All cases were both K27M and G34R/V wild type and most of them corresponded to glioblastomas (80%). Compared with IDH/H3 wild-type gliomas, H3 K27M-mutant gliomas were diagnosed at an earlier age (32 vs 64 y, $P < .001$), always had a midline location (20/20 vs 21/130, $P < .001$), less frequently had a methylated *MGMT* promoter (1/20 vs 52/129, $P = .002$), and lacked *EGFR* amplification (0/21 vs 26/128, $P = .02$; Table 2). These differences remained significant when H3 K27M-mutant gliomas were compared with the subgroup of IDH/H3 wild-type glioblastomas (median age: 32 vs 64 y, $P < .001$; *MGMT* promoter methylation 1/20 vs 44/105, $P < .001$; *EGFR* amplification 0/21 vs 24/106, $P < .001$). Compared with the subgroup of midline IDH/H3 wild-type gliomas (n = 21: glioblastoma n = 9, anaplastic glioma n = 6, low-grade glioma n = 6), H3 K27M-mutant gliomas were also diagnosed at an earlier age (32 vs 52 y, $P = .0006$), less frequently had a methylated *MGMT* promoter (1/21 vs 8/20, $P = .008$), and lacked *EGFR* amplification (0/21 vs 4/19, $P = .04$). Despite their younger age at diagnosis, no significant difference was observed between the median survival of H3 K27M-mutant and IDH/H3 wild-type gliomas (19.6 mo vs 17 mo, $P = .3$) or between H3 K27M-mutant and IDH/H3 wild-type midline gliomas (19.6 vs 32 mo, $P = .4$, Fig. 3).

Discussion

Three main subgroups of adult diffuse gliomas can be distinguished based on 1p/19q codeletion and the IDH mutation status.[17,18] Gliomas with the 1p/19q codeletion display the best prognosis, whereas the *IDH*-mutated gliomas, without 1p/19q codeletion, have an intermediate prognosis, and the non–1p/19q codeleted and non–IDH-mutated gliomas have a poor prognosis. However, the latter group remains heterogeneous and it has been suggested that IDH wild-type gliomas may be further classified based on the *TERT* promoter mutation status,[14,17] gene expression, methylation, and genomic profiling.[13,19] In addition, a subset of these gliomas harbors histone mutations.[13] In the

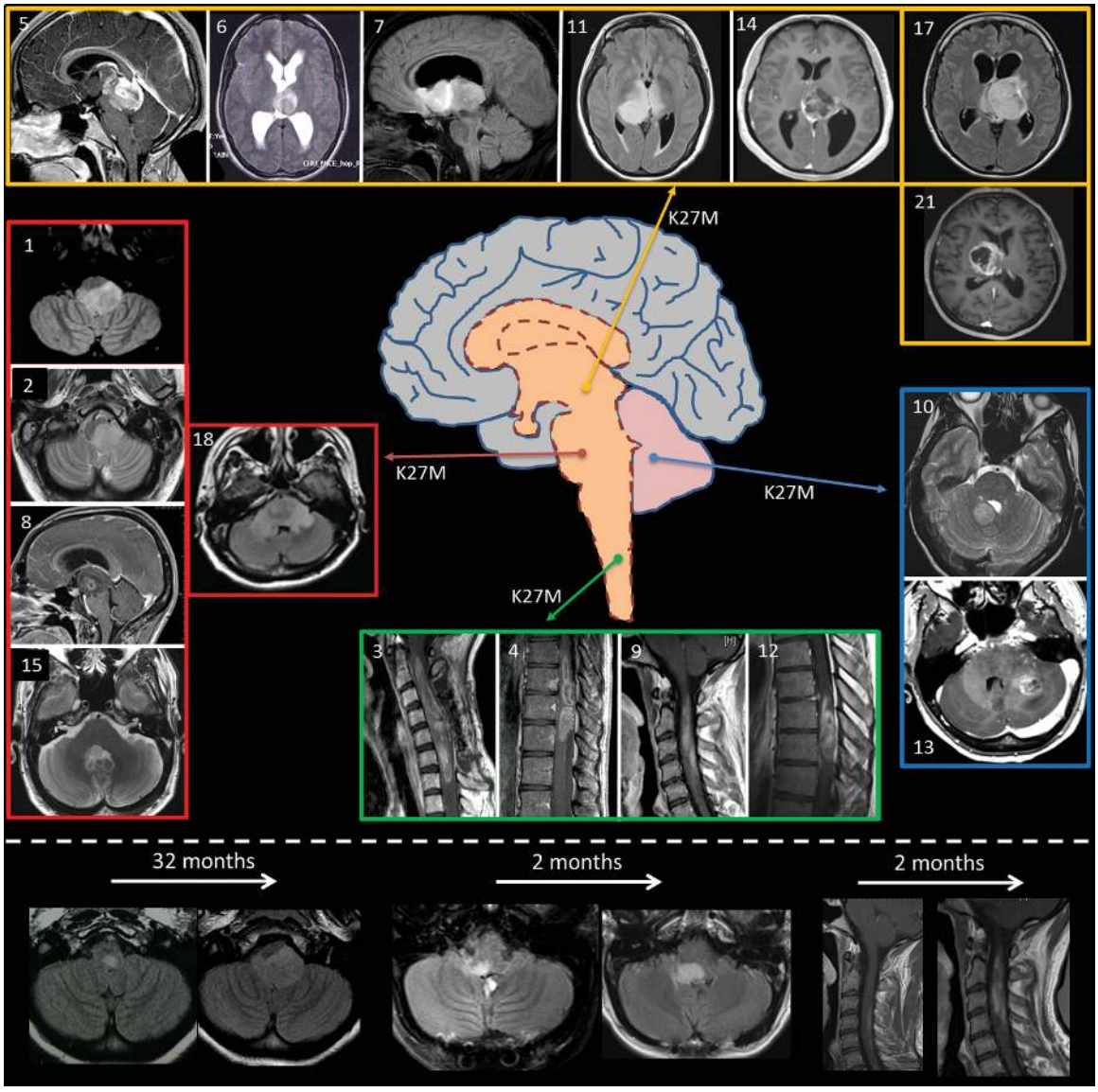

Fig. 1 MRI characteristics of adults with H3 K27M-mutant gliomas. Top: numbers correspond to the patient numbers. For patients 1, 2, 6, 7, 10, 11, 15, 17, and 18, who had non-enhancing tumor, axial T2/fluid-attenuated inversion recovery (FLAIR) sequences are shown. For patients 3, 4, 5, 8, 9, 12, 13, 14, and 21, who had contrast-enhancing tumor, T1 post-contrast sequences are shown. Bottom: tumor growth before treatment onset in patients 1, 15, and 9. Patient 1 presented with unexplained anorexia and vomiting 32 months before diagnosis that led to an MRI that was considered normal at the time despite the presence of a hypersignal within the medulla oblongata. In patient 15, initial suspicion of grade I ganglioglioma led to an initial follow-up. In patient 9, initial follow-up resulted from the initial suspicion of a myelitis.

present study, we found that H3 K27M mutations define a distinct subgroup of adult IDH wild-type diffuse gliomas and that determining the H3 K27M mutation status seems particularly important for appropriately diagnosing and grading adult IDH wild-type midline gliomas.

Though the occurrence of H3 K27M-mutant gliomas in adults had been previously reported,[7,9–13] their characteristics had not been specifically described. As observed herein, adult H3 K27M-mutant gliomas predominate in patients aged <40 years, but they can occur at any age, even in elderly patients. These gliomas seem to be rare in the entire population of adult IDH wild-type diffuse gliomas but relatively frequent among the subset of these tumors with midline location. In 2 series of 160 and 415 adult IDH wild-type astrocytomas, H3 K27M mutations were identified in only 9 (7.5%) and 10 tumors (2%), respectively.[13,20] However, in 4 studies of adult diffuse midline gliomas, they were detected in 7 out of 15 spinal gliomas (46%), in 10 out of 20 and 10 out of 15 thalamic gliomas (50% to 66%), as well as in 3 out of 8 and 15 out of 28 brainstem gliomas (37.5% to 53%).[9–12]

In the present series, and as reported elsewhere,[7,9–13] the characteristics of adult H3 K27M-mutant gliomas were very similar to those reported in the pediatric population.[3–7] Their constant midline location suggests that in adults, as in children, H3 K27M mutations are specifically oncogenic in progenitors implicated in the development of

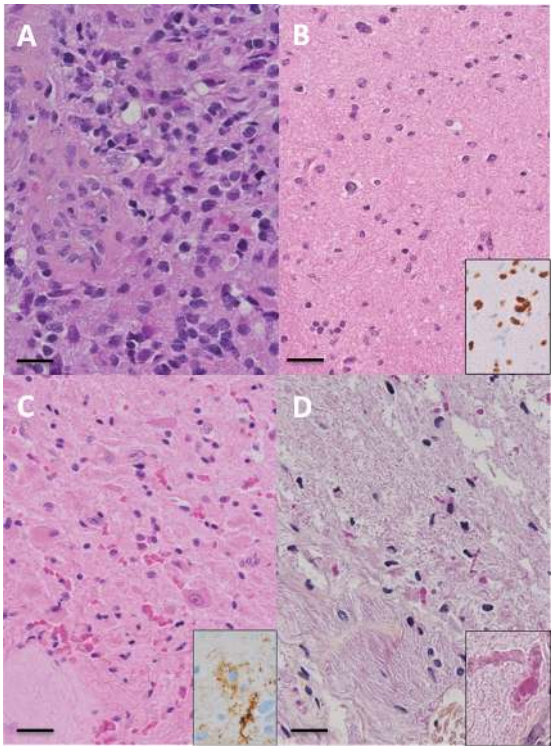

Fig. 2 Examples of histopathological features observed in adults with H3 K27M-mutant gliomas. Hematoxylin and eosin (H&E) × 400 (scale bar = 20 µm). (A) Histopathological features of case 9 diagnosed as spinal glioblastoma showing a highly pleomorphic infiltrating astrocytoma with microvascular proliferation. (B) Histopathological features of case 11 initially diagnosed as a thalamic low-grade astrocytoma showing a tumor of low density with astrocytic neoplastic cells and p53 staining (embedded picture). (C) Histopathological features of case 12 initially diagnosed as a spinal ganglioglioma showing a tumor of low density with neoplastic glial and ganglionic tumor cells expressing CD34 (embedded picture). (D) Histopathological features of case 1 initially diagnosed as a brainstem pilocytic astrocytoma showing a low density with compacted bipolar cells intermingled with Rosenthal fibers (shown at a higher magnification in embedded picture).

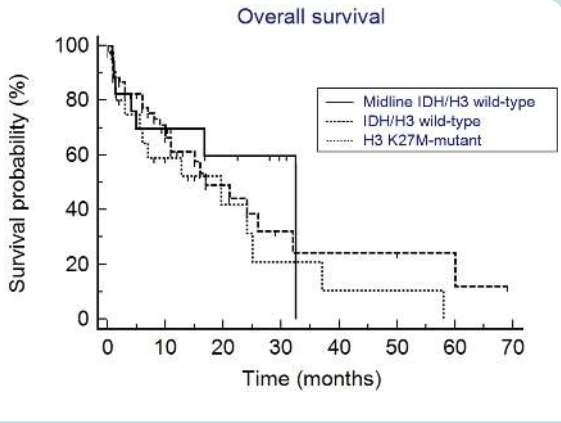

Fig. 3 Overall survival in patients with H3 K27M-mutant gliomas, IDH/H3 wild-type gliomas (irrespective of localization), and midline IDH/H3 wild-type gliomas.

midline structures.[21] Nevertheless, the specific location on the midline seems to vary with age. In children, H3 K27M-mutant gliomas are frequently located within the pons; in adults, as observed here, they seem more frequently located in the thalamus and in the spine.[7] In children, *H3F3A* K27M mutations are approximately 3 times more frequent than *HIST1H3B* K27M mutations, occur in older children, and are associated with an even poorer prognosis[22]; herein, all but one patient had an *H3F3A* K27M mutation suggesting that *HIST1H3B* K27M mutations are much less frequent in adults than in children. As reported in children, we found that in adults, H3 K27M mutations were frequently associated with TP53 overexpression and are exclusive with *IDH* mutation,[3] *EGFR* amplification,[23] and *MGMT* promoter methylation.[23] *TERT* promoter mutations also seem to be rare in these tumors. This finding is consistent with the fact that telomere maintenance in H3 K27M-mutant gliomas seems to result mainly from alternative lengthening of telomeres through *ATRX* mutations.[24] The rate of *TERT* promoter mutations was not determined in the present cohort of IDH/H3 wild-type gliomas, yet previous series have reported a 70% to 80% mutation rate in IDH wild-type diffuse gliomas,[13,20] suggesting that, as *MGMT* promoter methylation and *EGFR* amplification, these mutations are also much less frequent in adult H3 K27M-mutant than in IDH/H3 wild-type gliomas. In children, additional alterations in H3 K27M-mutant gliomas include amplifications of *PDGFRA* (30%), *CDK4/6* or *CCND1-3* (20%) or *MYC/PVT1* (15%) and mutations of *ACVR1* and *PPM1D* (15% to 20%).[4,25,26] Mutations involving alterations in TP53 cell-cycle (*TP53/PPM1D*) and in specific growth factor pathways (*ACVR1/PIK3R1*) seem to be particularly important for tumorigenesis.[27] The frequency of these alterations remains to be determined in adult H3 K27M-mutant gliomas.

Interestingly, in nearly half of patients, the histopathological presentation was not primarily suggestive of a high-grade diffuse glioma, and the identification of an H3 K27M mutation had a major impact on diagnosis. In 3 patients, initial analysis suggested a diffuse low-grade glioma. After molecular analysis, these patients were reclassified as WHO grade IV. Indeed, because grade has not been shown to predict outcome in H3 K27M-mutant gliomas, these gliomas correspond to grade IV even when mitotic figures, microvascular proliferation, or necrosis is not observed.[8] In a recent study of both pediatric and adult cases, 21% of H3 K27M-mutant gliomas had a histopathological aspect that was suggestive of a diffuse low-grade glioma,[7] and in previous studies of adult H3 K27M-mutant gliomas, the rate of tumors suggestive of a diffuse low-grade glioma was 14% in spinal gliomas (1 out of 7 cases) and ranged from 0 to 30% in thalamic gliomas (0 out of 11, and 3 out of 10 cases) and from 0 to 53% in brainstem gliomas (0 out of 3, and 8 out of 15 cases).[9–12] In 7 patients it was also difficult to distinguish between a diffuse and a circumscribed glioma. This difficulty likely resulted from the fact that only small biopsies could

be analyzed in most cases and from the histological heterogeneity of H3 K27M-mutant gliomas. As recently reported, H3 K27M-mutant gliomas can display a broad spectrum of histological features, including giant, epithelioid, and rhabdoid cells; primitive neuroectodermal tumor–like foci; ependymal-like areas; sarcomatous transformation, as well as features that may wrongly suggest circumscribed gliomas such as neuropil-like islands, pilomyxoid features, ganglionic differentiation, and pleomorphic xanthoastrocytoma-like areas.[7] Rarely, H3 K27M mutations have also been described in gliomas with typical features of circumscribed gliomas or with BRAF alterations as typically observed in pediatric low-grade gliomas. In children, H3 K27M mutations have been reported in 4 cases of typical midline gangliogliomas with a BRAFV600E mutation,[28–30] in 2 cases of spinal and thalamic pilocytic astrocytomas,[31,32] and in 4 cases of midline unclassified gliomas that were associated with a BRAFV600E mutation.[32,33] In adults, H3 K27M mutations have been reported in 2 cases of spinal and cerebellar anaplastic gangliogliomas.[11,30] It is currently unknown how these tumors should be classified. The very poor prognosis of some patients suggests that some of these cases may correspond to atypical high-grade diffuse H3 K27M-mutant gliomas.[28,33] Yet the prolonged survival observed in other patients (up to 10 y)[31] suggests that some cases may correspond to an overlap between circumscribed and H3 K27M-mutant gliomas and that not all gliomas with an H3 K27M mutation may correspond to grade IV.[32]

The median survival in children with diffuse H3 K27M-mutant gliomas is typically less than 1 year, with a 2-year survival rate of <10%. In adults, these gliomas also seem to be associated with a poor prognosis. In the present series, the median survival was 19.7 months, which is consistent with a median survival of 16.9 months,[13] 10.4 months,[9] and ~16 months[10] reported in previous series of adult H3 K27M-mutant gliomas. Most patients reported herein who were given an oncological treatment received temozolomide radiochemotherapy. However, the benefit of temozolomide in these patients is uncertain. In pediatric brainstem gliomas, which are mostly H3 K27M-mutant, temozolomide radiochemotherapy has not been shown to improve overall survival compared with radiotherapy alone, which is possibly due to the very low rate of *MGMT* promoter methylation in these tumors.[34] There is significant hope that therapeutic progress will result from the important advances achieved in understanding H3 K27M-mutant glioma pathogenesis.[24,35,36] Histones package DNA into chromatin, and their epigenetic modifications regulate the chromatin state and gene expression. The K27 residue is critical for all histone H3 variants; K27 methylation (mono, bi, or trimethylation) has an inhibitory effect on gene expression, while K27 acetylation activates transcription. The H3 K27M mutation, which is a very early event in these gliomas and is found throughout the tumor,[27] leads to a global downregulation of K27 methylation on all H3 molecules, either wild-type or mutated, even though the mutated H3 protein represents only ~5% of the total H3 in the cell.[37–41] This effect results from a biochemical inhibition of polycomb repressor complex 2 (PRC2), the major H3 K27 methylase, by K27M-mutant histones.[37,40] The aberrant gene expression resulting from PRC2 inhibition is further reinforced by DNA hypomethylation.[37,41] Recently, in 2 preclinical studies, the small molecule inhibitor GSKJ4 of the histone demethylase JMJD3 and the histone deacetylase inhibitor panobinostat have been shown to increase the K27M methylation levels and to have potent antitumor efficacy in H3 K27M-mutant cell lines and xenografts.[35,36] These findings suggest epigenetic modulators as promising treatments in H3 K27M-mutant gliomas.

The limitations of the present study include its retrospective design and small sample size, the absence of direct comparison with a pediatric series, as well as the absence of large-scale molecular analysis precluding the identification of potential age-related molecular characteristics in H3 K27M-mutant gliomas. Future studies with larger cohorts, including both pediatric and adult cases, are warranted to confirm the data presented herein, complete the description of H3 K27M-mutant glioma characteristics, and potentially refine the classification of these tumors. Nevertheless, we show that H3 K27M mutations in adults, as in children, define a distinct subgroup of IDH wild-type gliomas that are characterized by a constant midline location, low rate of *MGMT* promoter methylation, and poor prognosis. The potentially misleading histological presentation of these tumors supports assessing H3 K27M mutations in adult IDH wild-type midline gliomas to ensure appropriate diagnosis and grading. In the future, assessing H3 K27M mutations may also enable patients to participate in clinical trials using dedicated therapeutic strategies.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

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