Neuro-Oncology Advances

7(1), vdaf200, 2025 | https://doi.org/10.1093/noajnl/vdaf200 | Advance Access publication September 9, 2025

Pediatric H3 K27-altered diffuse midline gliomas may consist of two clinically relevant subsets based on patient age and molecular genetic profile

Yufan Yang, Nitin Wadhwani, Yuki Aoi, Sachin Govind, Sean Sachdev, Ali Shilatifard, Daniel Brat, Roger Stupp, and Michael DeCuypere

All author affiliations are listed at the end of the article

Corresponding Author: Michael DeCuypere, MD, PhD, Division of Pediatric Neurosurgery, Ann & Robert H. Lurie Children's Hospital of Chicago, Assistant Professor of Neurological Surgery, Northwestern University Feinberg School of Medicine, 225 East Chicago Avenue, Box 28, Chicago, IL 60611-2991, USA (mdecuypere@luriechildrens.org)

Abstract

Background. Diffuse midline gliomas, H3 K27-altered (DMG), are rare CNS WHO grade 4 tumors characterized by the global loss of K27me3 on histone H3. DMGs cannot be safely resected and are associated with poor outcomes in children, though relatively few DMGs have undergone extensive molecular and clinical profiling. Herein, we describe the clinical and molecular profiles of 36 pediatric DMGs as they relate to patient outcomes.

Methods. Pediatric patients (<18 y/o) between 2015 and 2024 with biopsy-proven DMGs and next-generation sequencing (NGS) panels of 86 hotspot genes were reviewed for clinicopathologic characteristics and survival outcomes. Gene Ontology (GO) enrichment analysis was performed. Progression-free survival (PFS) and overall survival (OS) were calculated according to the Kaplan-Meier method. Multivariate Cox regression analysis was performed.

Results. Thirty-six patients were included (median age = 9 y/o). Patients <10 y/o at diagnosis ($n = 24$) progressed significantly earlier and experienced significantly greater mortality than patients ≥10 y/o at diagnosis ($n = 11$); PFS and OS at 12 months were 9.0% and 26% for <10 y/o and 36% and 64% for ≥10 y/o (PFS: $P \leq .03$, OS: $P < .03$). NGS findings revealed *PIK3CA* mutations occurred only in patients <10 y/o (10/25), and GO analysis revealed patients <10 y/o were significantly more enriched for *PI3K/AKT* signaling pathway alterations than patients ≥10 y/o ($P \leq .03$).

Conclusion. We present findings that suggest "adolescent" DMGs carry more favorable prognosis and are molecularly distinct from earlier onset pediatric DMGs. These findings have implications in the design and interpretation of clinical trials, in addition to informing clinical practice.

Key Points

- DMG patients <10 y/o have worse survival outcomes compared to those ≥10 y/o
- DMG patients <10 y/o have significantly greater PI3K/AKT mutation frequency than those ≥10y/o
- Adolescent age should be considered in DMG clinical trial design and interpretation

Received March 26, 2025; accepted September 04, 2025.

Importance of the Study

Given their proximity to eloquent CNS structures and location commonly in the brain stem, these tumors were historically diagnosed and treated based on clinical characteristics. Only recently have stereotactic biopsies of infiltrative midline lesions become a more common practice, and few DMGs have been extensively characterized by modern molecular diagnostics. We describe a large pediatric DMG series with comprehensive molecular and clinical profiling and provide evidence that "adolescent" DMGs may be distinct based on molecular profile and clinical behavior. These findings may change how clinical trial data is interpreted and lead to adolescent age being accounted for in clinical trial design/recruitment (ie being a confounder when evaluating the efficacy of novel treatments). Our cohort revealed significantly prolonged PFS and OS among adolescent patients 10 years or older, compared to young children under the age of 10 years. We further describe significantly increased frequency of *PI3K/AKT* pathway alterations in patients <10 y/o.

Diffuse Midline Gliomas, K27-altered (DMGs), are rare CNS World Health Organization (WHO) grade 4 tumors classified under the pediatric-type diffuse high-grade gliomas in WHO CNS5.[1] These lesions have hallmark characteristics on neuroimaging and are localized to the diencephalon, brainstem, and cervical spine. DMGs mainly affect young children and are associated with poor clinical outcomes, with a median overall survival (OS) of ≤12 months.[2-5] DMGs are diffusely infiltrative tumors and cannot be safely resected, particularly those that present in the pons/brainstem or thalami.[6-8] DMGs additionally demonstrate inherent resistance to other treatments such as radiotherapy (RT) and chemotherapy—although focal RT is the current standard of care.[9-13] The key driver of tumorigenesis in DMGs involves the substitution of lysine for methionine (K27M) in histone proteins H3.1 or H3.3 and/or a global reduction of trimethylation at lysine 27 on histone H3 (H3K27me3), leading to pathologic transcription at specific genomic sites.[14-26] Clinical outcome studies have suggested that adult DMGs are associated with improved prognosis compared to pediatric DMGs, which may be partially due to higher rates of cervical spine localization that portend better survival; older children, or "adolescent" DMGs, may have better survival than younger children as well, but the survival of adolescent DMGs has yet to be explicitly studied in an exclusively DMG coh ort.[2,4,6,27-34] However, clinical understanding of DMGs—a biopsy-proven molecular diagnosis—is lacking compared to that of diffuse intrinsic pontine glioma (DIPG), which is diagnosed based on neuroimaging and may include molecularly heterogenous lesions (likely 80%-90% DMGs) confined to the pons. Stereotactic biopsies of the midline brain and brainstem were discouraged until recent years due to the belief of risk outweighing the benefits and lack of therapeutic consequences.[4,11,13,20,35,36] In settings where stereotactic biopsy is not feasible, there has been an increased reliance on liquid biopsy via the sampling of CSF for K27M circulating tumor DNA in conjunction with clinical and radiologic data to reach a diagnosis.[37] Molecular profiling of biopsy-proven DMGs with clinical and treatment correlations may add to our understanding and offer further age-specific or molecular prognostic stratification.[3-5,28,32,38] To inform current and planned DMG clinical trials assessing specific systemic agents, targeted therapies, and applications of RT, we present a large institutional pediatric DMG cohort with molecular genetic profiling, treatment descriptions, and clinical characteristics.

Materials and Methods

The protocol for this retrospective analysis and data collection, including patient demographics, treatments, and outcome analyses, was approved by the Institutional Review Board of the Ann and Robert H. Lurie Children's Hospital of Chicago (IRB #LCH2022-5620).

We examined electronic medical records and retrospectively reviewed all children at Lurie Children's Hospital diagnosed with a brain tumor and had the term "grade 3" or "grade 4" in the pathology report between the years 2015-2024. From an initial list of 731 pediatric brain tumor patients, we manually identified patients with biopsied, pre-treatment tumors that had a documented "K27M" mutation or DMG *EZHIP* or *EGFR* subtype diagnosis and available next-generation sequencing (NGS) data. Our search strategy encompassed patients with low-grade histology (grade 2) that received a final integrated molecular diagnosis of "Diffuse Midline Glioma, H3 K27-altered" or "Diffuse Midline Glioma, H3 K27-mutant (WHO CNS4)," as these diagnoses included "WHO grade 4" classifications on pathology reports—with rare "WHO grade 3/4" classifications on pre-2018 pathology reports. NGS at Lurie Children's Hospital entails DNA sequence analysis on 86 genes for hotspot regions, 44 genes for full coding regions, and 28 genes for focal high-level copy number gains. Identified patients underwent further data collection, including clinical course, treatments received, and molecular/histologic characteristics. Tumor location was noted as the primary anatomical CNS region of tumor origin. Radiation-related toxicities were documented and assessed by the Radiotherapy Therapy Oncology Group toxicity scale (RTOG) based on descriptive information provided in follow-up notes from an attending radiation oncologist. Acute RTOG Grade I toxicity is defined as minor neurological findings that do not require medication; Grade II toxicity is defined as neurological findings sufficient to require home-nursing care and/or medication; Grade III toxicity is defined as neurological findings requiring hospitalization; Grade IV toxicity is defined as severe neurological impairment, including paralysis, coma, and seizures >3 times a week despite medication and hospitalization.[39]

The primary endpoints were progression-free survival (PFS) and overall survival (OS). PFS was defined as the elapsed duration between the date of completed initial

intervention and the date of first radiographic and/or symptomatic evidence of tumor progression. Tumor progression was based on clinical examination findings from the attending neuro-oncologist that showed evidence of neurological deterioration requiring treatment changes, as well as a corroborating MRI.

OS was defined as the elapsed duration between the date of biopsy and the date of death or last clinical contact. OS after first progression was defined as the elapsed duration between the date of first progression and date of death or last clinical contact.

Gene Ontology

Reactome pathway over-representation analysis for 31 mutated genes found in the cohort was performed using the ReactomePA package in R.[40] The top 15 pathways were visualized as a dot plot.

Statistical Analysis

GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used to perform summary statistics and analyses. PFS and OS were calculated according to the Kaplan-Meier (KM) method. Log-rank tests were utilized to assess for statistical significance between Kaplan-Meier (KM) curves (performed by YY). All subsequent statistical analyses were performed using R (version 4.4.2). Statistical significance was defined as a *P*-value of ≤.05.

A box plot comparing the enrichment of *PI3K/AKT* pathway alterations (*PDGFRA/PIK3CA/MET/PTEN/EGFR/KIT/PTPN11* mutations) in patients <10 y/o and patients ≥10 y/o was performed, with the Mann-Whitney U test being used to assess for statistical significance; a scatter plot showing the distribution of age of onset, OS, and *PI3K/AKT* pathway alterations was generated (performed by YA). Multivariate Cox regression analysis was performed and was verified for the assumption of proportional hazards using standard diagnostic tests (performed by YY).

Results

Cohort Characteristics

A summary of patient, tumor, and treatment characteristics can be found in Table 1. We identified 36 molecularly sequenced DMG patients that had a median age of 8 years (range = 2-17 years). There were 25 patients < 10 years old (y/o) and 11 patients ≥10 y/o. The pons and/or brainstem was the most frequent location (72%), with the thalamus being the second most frequent (22%) and the cervical spine being the least (6%). The majority of primary thalamic DMGs were found in patients <10 y/o, while cervical spine manifestation only occurred in patients ≥10 y/o. Most patients only received a diagnostic biopsy (78%); among patients that received surgical debulking (22%), 2/8 were thalamic lesions, and both cervical spine lesions were debulked. All patients but one—a patient that did not receive any RT or systemic treatment due to personal wishes—received RT as their primary treatment, either focal external beam radiotherapy or craniospinal radiotherapy. The patient that did not receive any RT or systemic treatment was excluded from all survival analyses (See section "*Survival Measures of Cohort and Sub-groups*"). For progressive disease, re-irradiation (re-RT) was pursued in 12 patients, who received a median lifetime radiation dosage of 84 Gy (range = 74-106); patients that did not receive re-RT had a median lifetime radiation dosage of 54 Gy (*n* = 22, range = 0-58). Amongst patients <10 y/o, 9/25 (36%) received re-irradiation compared to 3/11 patients ≥10 y/o receiving re-irradiation (27%). Nearly all patients received some systemic therapy (92%), with 53% receiving more than one modality. For patients that received re-RT, 91% were administered some additional systemic therapy between completion of re-RT and last clinical contact and/or death. There were no acute radiation-attributed complications reported in our cohort, with all patients experiencing Grade I or less toxicities, as defined by the RTOG Common Toxicity Criteria.[39] Figure 1A describes individual patient clinical and treatment profiles, with patients numbered based on decreasing OS, and the primary systemic modality for each patient received is noted.

Molecular Characterization of Cohort

Pathology and NGS reports from tumor biopsy samples were available for all 36 patients. Histology reports for 32 patients were classified as "high-grade" (grade 3 or grade 4), while 4 patients were classified as "low-grade" (grade 2). Diagnosis-establishing *H3F3A* (histone H3.3 encoding gene) and *HIST1H3B* (histone H3.1 encoding gene) mutations were identified in 29 patients and 5 patients, respectively. Two patients did not have a mutation in *H3F3A* or *HIST1H3B* but instead were *EGFR*-altered subtypes of DMG based on DNA methylation and NGS findings (still considered H3 K27-altered). Figure 1B details the molecular profiles for each patient, with corresponding tumor location, Ki67 proliferation value, and OS with age stratification between patients <10 y/o and patients ≥10 y/o. *TP53* was the most frequently mutated gene, occurring in 58% of lesions. Alterations in *PIK3CA* (28%), *PDGFRA* (19%), *NF1* (14%), and *ACVR1* (14%) were the next most frequent. Stratifying molecular profiles based on age <10 y/o and ≥10 y/o (Figure 1B) revealed that *PIK3CA* alterations were only present in patients <10 y/o for, and 6/10 *PIK3CA* mutations did not co-occur with *HISTH13B* and/or *ACVR1* mutations. Among patients <10 y/o, 40% had a *PI3KCA* alteration (10/25) compared to none of the patients ≥10 y/o (0/11) having one. GO analysis further revealed that, while our cohort overall was enriched for *PI3K/AKT* pathway alterations, patients <10 y/o had a significantly greater number of *PI3K/AKT* pathway alterations compared to patients ≥10 y/o (Figure 2B, *P* < .03). GO analysis additionally showed cohort enrichment for *TP53* and *MAPK* signaling pathway alterations (Figure 2A). Alterations in the *TP53* signaling pathway (*TP53* and/or *PPM1D* mutations) occurred in 74% of patients <10 y/o (17/23) and 55% (6/11) of patients ≥10 y/o. Among those patients with longer OS (top 50%), 44% (8/18) had a *TP53* alteration; among patients with shorter OS (lower 50%),

Table 1. General characteristics

Overall cohort (n=36)		Patients <10 y/o (n=25)		Patients ≥10 y/o (n=11)	
Variable	Number (%)	Variable	Number (%)	Variable	Number (%)
Age (years)		**Age (years)**		**Age (years)**	
Median	8	Median	7	Median	14
Range	2-17	Range	2-9	Range	10-17
Tumor location		**Tumor location**		**Tumor location**	
Pons/Brainstem	26 (72)	Pons/Brainstem	18 (72)	Pons/Brainstem	8 (73)
Thalamus	8 (22)	Thalamus	7 (28)	Thalamus	1 (9)
Cervical Spine	2 (6)	Cervical Spine	0	Cervical Spine	2 (18)
DMG molecular type		**DMG molecular type**		**DMG molecular type**	
H3F3A	29 (81)	H3F3A	18 (72)	H3F3A	11(100)
HIST1H3B	5 (14)	HIST1H3B	5 (20)	HIST1H3B	0
EGFR Sub-type	2 (5)	EGFR Sub-type	2 (8)	EGFR Sub-type	0
Surgery		**Surgery**		**Surgery**	
Biopsy	28 (78)	Biopsy	23 (92)	Biopsy	5 (45)
Debulking	8 (22)	Debulking	2 (8)	Debulking	6 (55)
Systemic therapy		**Systemic therapy**		**Systemic therapy**	
Yes	33 (92)	Yes	22 (88)	Yes	11 (100)
No	3 (8)	No	3 (12)	No	0
Re-irradiation		**Re-irradiation**		**Re-irradiation**	
Yes	12 (33)	Yes	8 (32)	Yes	3 (27)
No	24 (67)	No	17 (68)	No	8 (73)
RT toxicity		**RT Toxicity**		**RT Toxicity**	
≤Grade I	36 (100)	≤Grade I	25 (100)	≤Grade I	11 (100)
>Grade I	0	>Grade I	0	>Grade I	0

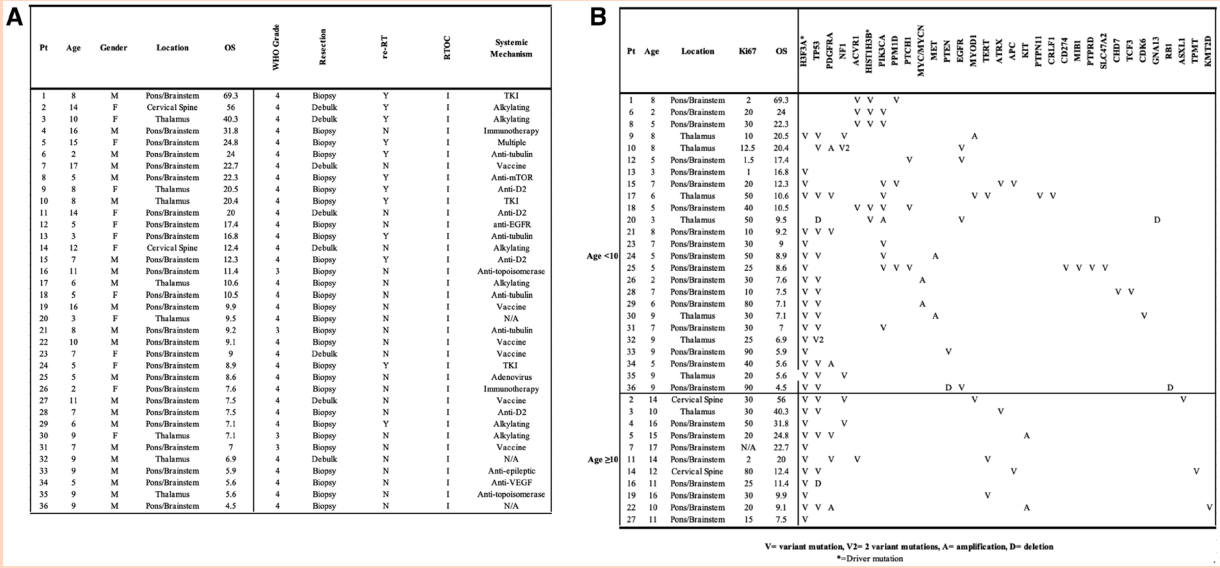

Figure 1. Individual patient profiles based on treatment characteristics (A) and mutational composition based on NGS (B).

72% (13/18) had a *TP53* alteration. The only *MAPK* signaling pathway alterations that occurred in our cohort were *NF1* mutations, involving 13% (3/23) of patients <10 y/o and 18% (2/11) of patients ≥10 y/o. *NF1* alterations demonstrated a locational preference, with 4/5 *NF1*-mutant lesions presenting in non-pontine locations. Every *HISTH13B*-mutant DMG presented in patients <10y/o and had a co-occurring *ACVR1* mutation, with 3 out of 4 patients being the longest surviving in this age range. Recurring mutations in *PPM1D, PTCH1, MYC/MYCN*, and *MET* were only present in

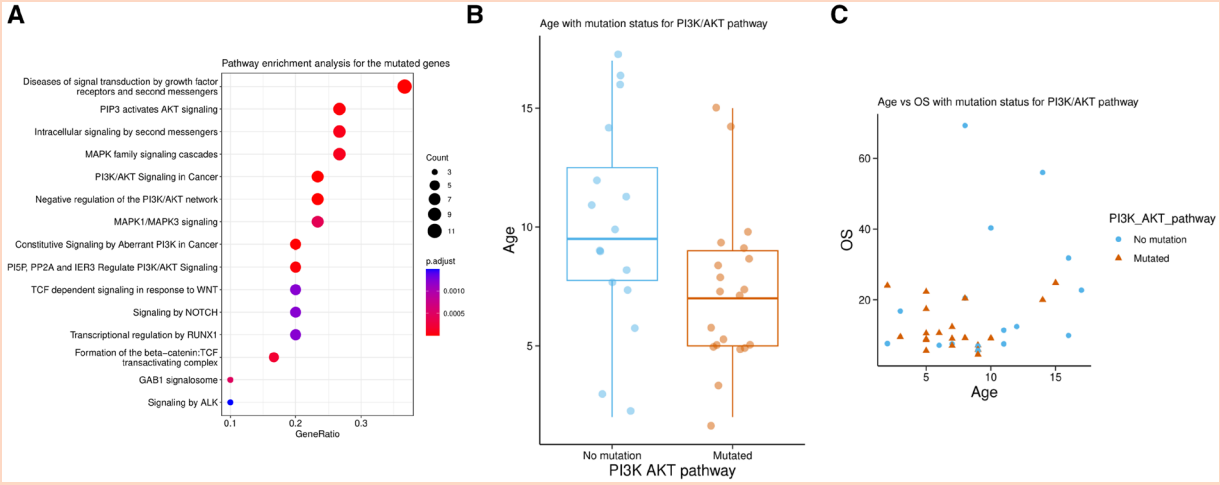

Figure 2. Gene ontology enrichment analysis of the cohort to identify recurrent intracellular mechanism disruption (A). Box plot comparing the frequency of *PI3K/AKT* pathway enrichment between patients age <10 y/o and ≥10 y/o (B). Scatterplot visualizing the distribution of *PI3K/AKT* pathway alterations with respect to age and OS (C).

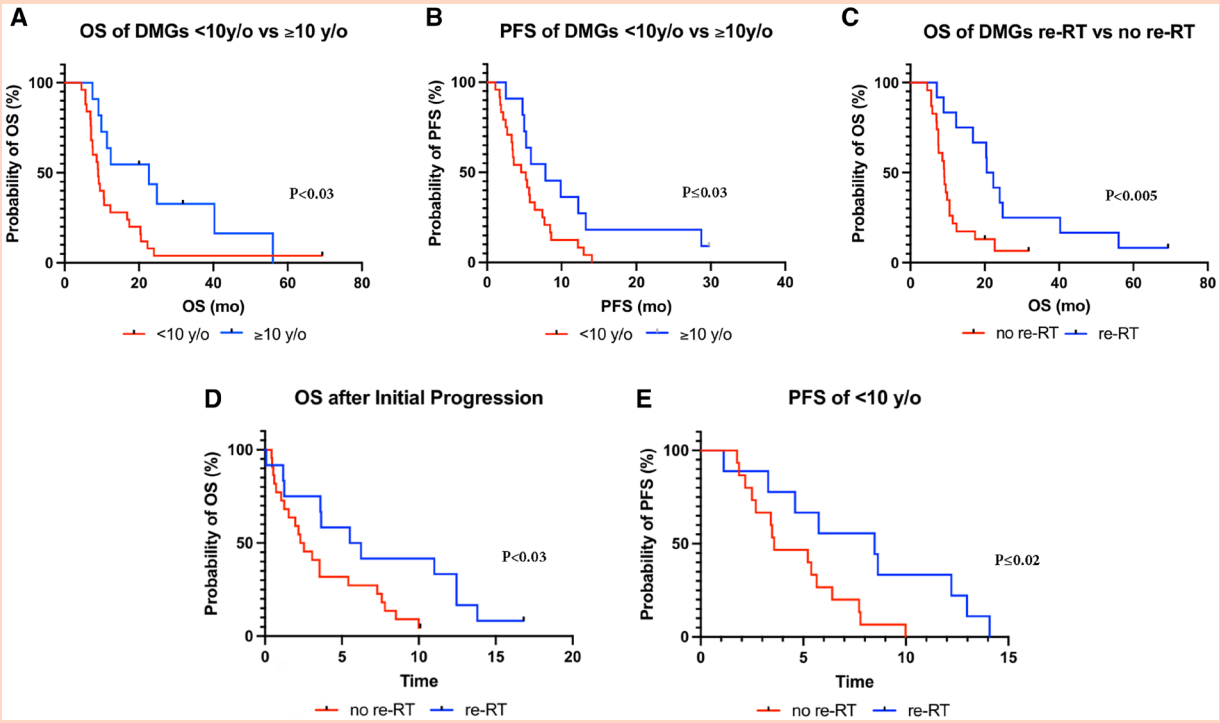

Figure 3. Kaplan-Meier survival curves comparing various sub-groups: OS of <10 y/o patients versus ≥10 y/o patients (A), PFS of <10 y/o patients versus ≥10 y/o patients (B), OS of patients that received re-irradiation (reRT) and those that did not (C), OS after first progression of patients that received re-irradiation versus those that did not (D), PFS of only patients <10 y/o that received re-irradiation and those did not (E).

DMGs <10 y/o. The only recurring mutations exclusive to DMGs ≥10 y/o were *KIT* alterations, and both cases co-occurred with *PDGFRA* mutations.

Survival Measures of Cohort and Sub-Groups

Median PFS and OS for the cohort were 5.4 (95% CI: 3.4-7.7) and 10.5 months, respectively (95% CI: 5.7-15.2). Median OS based on tumor location was as follows: 9.6 months (95% CI: 6.9-17.1) for lesions of the pons/brainstem (n=26), 10.6 months (95% CI: 4.0-20.2) for lesions of the thalamus (n=7), and 34 months for lesions of the cervical spine (n=2). Calculated probability of PFS and OS for the cohort at 12 months was 20% and 43%, respectively (Figure 3A). For sub-group analysis, the probability of PFS and OS was compared for the following categories: age <10 y/o versus age ≥10 y/o, age <10 y/o versus age ≥10 in pontine/brainstem lesions,

pontine/brainstem versus thalamus, re-irradiation versus no re-irradiation, biopsy versus debulking, *TP53*-mutant versus *TP53*-WT, and *PIK3CA*-mutant versus *PIK3CA*-WT. Probability of OS after first progression for those that received re-irradiation and those that did not was additionally assessed.

Patients <10 y/o had significantly decreased PFS and OS compared to those ≥10 y/o (Figure 3A and B). Probability of OS at 12 months was 26% for <10 y/o patients compared to 64% for ≥10 y/o patients (*P*-value < .03, HR: 2.2, 95% CI: 1.1-4.6). When isolated to pontine lesions, patients <10y/o did have improved probability of OS at 12 months compared to patients ≥10 y/o (24% vs 50%), but this finding was not statistically significant (*P*-value = .09, HR: 2.2, 95% CI: 0.9-5.0). Median OS for patients <10 y/o was 9.1 months (95% CI: 5.4-16.1), and median OS for patients ≥10 y/o was 22.7 months (95% CI: 11.0-29.0). This significant difference in OS was not observed when comparing patients <9y/o and ≥9 y/o, with the probability of OS at 12 months being 35% and 40.0%, respectively (*P*-value < .4, HR: 1.4, 95% CI: 0.68-2.8). Patients that received re-irradiation had a greater probability of OS compared to those that did not, with a 12-month OS of 75% compared to 17% (Figure 3C, *P*-value ≤ .005, HR: 2.5, 95% CI: 1.2-5.2). OS survival after first progression was additionally statistically significant between those that received re-irradiation and those that did not (Figure 3D, *P*-value <.02, HR: 0.45, 95% CI: 0.22-0.93). PFS between those that received re-irradiation and those that did not was not statistically significant across the whole cohort (*P*-value < .08, HR: 1.9, 95% CI: 0.94-3.8) or amongst patients ≥10 y/o (*P*-value < .5, HR: 0.57, 95% CI: 0.17-2.0) but was statistically significant when isolated to patients <10 y/o (Figure 3E, *P*-value < .01, HR: 3.8, 95% CI: 1.4-10.1).

Pontine/brainstem DMGs did not have significantly different actuarial survival outcomes compared to thalamic DMGs (*P*-value = .9, not shown). Patients that received diagnostic biopsy alone versus debulking also did not have significantly different OS (*P*-value < .2, not shown). Lesions with alterations in *TP53* and *PIK3CA* had a worse probability of 12-month OS than their WT counterparts (*TP53*: 53% vs 33%, *PIK3CA*: 44% vs 22%), but OS curves were not statistically significant (*P*-value= .2). A scatterplot of *PI3K/AKT* alterations plotted with respect to age of onset and OS revealed that *PI3K/AKT* alterations did not lead to noticeable changes in OS for patients <10 y/o but did decrease OS for patients ≥10 y/o (Figure 2C). Multivariate Cox regression analysis of several potential predictors of OS (Table 2) found age at diagnosis <10 y/o, *TP53* mutation, re-irradiation, and thalamic tumor location to be significant. Of these significant variables, only age at diagnosis and re-irradiation were also found to be significant on univariate log-rank test comparisons of Kaplan-Meier survival curves. Age at diagnosis <10 y/o carried the highest hazard ratio, with a 9 times greater risk of worse OS than age at diagnosis ≥10 y/o. *TP53* mutations were equally divided amongst patients age <10 y/o (60%) and age ≥10 y/o (55%), while more patients <10 y/o received re-irradiation and had thalamic tumors than patients ≥10 y/o (Table 1). *PI3K/AKT* pathway mutations, surgical debulking, and cervical spine location (likely due to low number) were not found to be significant predictors.

Table 2. Multivariate cox regression analysis

Variable	Mean OS ± SEM (months)	HR (95% CI)	*P*-value
Age at diagnosis			
<10 y/o	13.6 ± 2.7	8.97 (2.53-31.79)	.00068[a]
≥10 y.o	22.3 ± 4.6		
PI3K pathway			
PI3K mutation	12.3 ± 1.5	1.15(0.49-2.67)	.75
No PI3K mutation	21.7 ± 5.0		
TP53 status			
TP53-Mut	14.2 ± 2.9	7.79 (2.64-22.98)	.00020[a]
TP53-WT	19.2 ± 4.0		
Ki67			
≥30	14.7 ± 3.3	1.68 (0.76-3.73)	.2
<30	17.8 ± 3.8		
Resection status			
Biopsy	14.5 ± 2.4	2.47 (0.64-9.59)	.19
Debulking	24.0 ± 6.8		
Reirradiation			
no reRT	10.7 ± 1.3	7.44 (2.46-22.5)	.00039[a]
reRT	26.9 ± 5.5		
Location (vs pons)			
Spinal Cord	34.2 ± 21.8	0.13 (0.016-1.10)	.062
Thalamus	16.4 ± 4.0	0.26 (0.092-0.74)	.011[a]

Abbreviation: CI, confidence interval; HR, hazard ratio; SEM, standard error.
[a]Statistical significance.

Discussion

We provide evidence that there may be further subgrouping of pediatric DMGs based on age, as demonstrated by clinical and molecular differences between "adolescent" DMGs (age ≥10 y/o) and "child" DMGs (age <10 y/o) in our cohort. Adolescent DMGs (ages ~10-17 y/o) have rarely been identified as their own subgroup in the literature, often being grouped together with all DMGs <18 y/o as "pediatric DMGs" and do not receive demographic considerations in clinical trial recruitment.[4-6,27,29,38] Adolescent DMGs had statistically greater survival, corroborating a report made by the International DIPG registry, which found patients >10 y/o had greater OS in a cohort of mostly radiographically diagnosed "DIPGs," a clinical diagnosis given to pontine lesions that can be several different molecularly diagnosed tumors;[4] however, our findings were demonstrated in a smaller but entirely molecularly diagnosed cohort of "DMGs" and included lesions from the thalamus and cervical spine. In addition, we found that "child" DMGs had significantly greater enrichment for *PI3K/AKT* pathway mutations

compared to adolescent DMGs; previously, *PI3KCA* mutations were only described to be enriched in pediatric DMGs (<18 y/o) compared to adult DMGs (≥18 y/o) and did not identify differences in mutation frequency between younger and older pediatric patients, which we have done in our coh ort.[3,4,18,19,26,29,35] We additionally describe increased enrichment of alterations along the entire *PI3K/AKT* signaling pathway via GO in an age subset of pediatric DMG patients. Although the sample size is small, our study facilitates new discourse on the molecular and clinical understanding of DMGs.

Since histone tail modifications were first identified as driver mutations in pediatric high-grade gliomas, ushering in the age of tumor molecular characterization, there have been several updates to the molecular understanding of DMGs.[14,19,25,41] DMGs with *HISTH3B* K27M mutations have been shown to have longer survival, younger age of onset, and predominant pontine localization compared to tumors with driver *H3F3A* K27M mutations.[3,5,17-20] The finding that midline gliomas with *EGFR* mutations or *EZHIP* overexpression can also display uniform loss of H3K27me3 despite not possessing a K27M mutation led to the incorporation of these lesions into DMGs as an entity in the WHO CNS5.[1,15,23,42,43] Additionally, reports on adult DMGs (age ≥18 y/o) have provided support that they are associated with favorable clinical outcomes compared to pediatric DMGs (<18 y/o) and also have distinct mutational profiles.[2,6,27-29,32-34] However, outside of the International DIPG Registry report, few pediatric DMGs have been molecularly and clinically characterized to the extent of our cohort, positioning our study to look into prognostic insights and/or therapeutic avenues that have been relatively unexplored.[4] Patients <10 y/o in our cohort not only progressed significantly earlier after primary treatment ($P<.03$) and had significantly decreased OS compared to those ≥10 y/o ($P<.03$), but a greater proportion also received re-irradiation than adolescent patients ≥10 y/o (36% versus 27%). The *PI3K/AKT* intracellular signaling pathway is implicated in increased cell survival and division, and missense variants in catalytic subunit-encoding *PIK3CA* and inhibitor-encoding *PTEN* were only detected in our cohort amongst patients who were <10 y/o.[35,44] Compared to 48% of patients <10 y/o harboring a mutation in this pathway (10/*25 PIK3CA*, 2/25 *PTEN*), none of the 11 patients ≥10 y/o had one. *PIK3CA* mutations have been noted in a sizable percentage of DMGs (~12%), but their predilection for age <10 y/o has rarely been explicitly identified—although many known *PIK3CA*-mutant DMGs were <10 y/o when analyzing prior cohort characteristics.[3,4,18,19,26,35] *PIK3CA* mutations in DMGs were also believed to mostly co-segregate with *HIST1H3B* and *ACVR1* mutations, but 6/10 *PIK3CA*-mutant DMGs in our cohort did not have a corresponding *HIST1H3B* and/or *ACVR1* mutation.[3] Prior series that explored *PI3KCA* mutations in DMGs also did not utilize GO to show increased overall enrichment for the full spectrum of *PI3K/AKT* pathway alterations in a specific age group, which we demonstrated with child DMGs compared to adolescent DMGs ($P\leq.03$). Our findings are consistent with recent studies that utilized DNA methylation data to further identify prognostic sub-groups among DMGs, wherein DMGs with improved OS and higher frequency of *MAPK* signaling pathway alterations clustered together, while DMGs with worse OS and higher frequency of *TP53* signaling pathway alterations also clustered together.[28,32,33] In Stegat et al, DMGs in the improved survival *MAPK* cluster were comprised entirely of adolescents and adults, with no patient younger than 11 y/o, while DMGs in the worse survival *TP53* cluster were overall much younger, with a median age of 7.6 years.[28] In addition to reporting a *MAPK* cluster amongst a cohort comprised of long-term survivors (>36 months OS), Roberts et al., also reported a median cohort age of 13.6 y/o, further suggesting that the shift towards improved survival in DMGs happens in adolescence rather than adulthood.[32] Adolescent DMGs further appear to be set apart from child pediatric DMGs in terms of tumor location. In the literature, cervical spinal DMGs have predominantly presented in adults and an overwhelming majority of the few pediatric patients reported were >10 y/o, including all cervical spinal cases in a multi-institutional pediatric DMG cohort of 85 patients.[2,5,6,29–31,34] Our cohort supports this observation, with only patients ≥10 y/o having cervical spinal tumors. One theory on the reason adolescent DMGs may present with clinical, molecular, and anatomic differences as compared to younger pediatric DMGs is that the tumor microenvironment in the adolescent brain (ages 10-18) may be more similar to the adult brain (age >18) than the early child brain (age <10), facilitating immune-tumor cell interactions that lead to adolescent DMG tumor cells taking on cell states preferred by adult DMG tumor cells versus early child DMG tumor cells.[38,45]

There would be clinical implications if adolescent DMGs were to be accepted as a more favorable presentation of DMGs, akin to adult DMGs. This includes the interpretation of clinical trial data, with future trials having to account for adolescent age as a potential confounder when assessing the efficacy of an agent in pediatric patients—and something to be controlled for during patient recruitment and group assignment. Another implication is on the clinical decision-making level, as providers may feel more inclined to recommend location-specific but potentially beneficial clinical trials (ie GD2-directed CAR-T therapy) or re-irradiation as a secondary treatment to adolescent DMG patients, if adolescent DMGs indeed have longer PFS and OS.[46,47] The cohort in our study is not large enough to definitively establish adolescent DMGs as a subgroup having distinct prognostic considerations, but it is able to bring attention to the need for further study of adolescent DMGs and provide clinical value on a case-by-case basis.

Regarding the re-irradiation of DMGs, 35% of our cohort received re-irradiation following initial progression, and our findings corroborate prior reports that re-irradiation is a safe treatment route for progressive disease amidst concerns for radiation necrosis; however, there is a risk that toxicity symptoms are underreported, as it is based off progress notes for terminally ill patients.[10–12,46,47] Although re-irradiation is a readily available treatment avenue for progressive DMGs, there have been relatively few studies to assess the survival benefit of re-irradiation, and all prior studies were performed in mostly radiographically diagnosed DIPGs, not biopsy-proven DMGs.[12,47] Patients that received re-irradiation in our cohort did have significantly improved OS after progression compared to those that did not, but the true survival benefit of re-irradiation cannot be accurately ascertained due to many re-irradiated patients also receiving concurrent systemic therapies, the lack of a matched cohort, and the potential that patients who received re-irradiation

had intrinsic factors (ie tumor pathology) that predisposed them for longer survival.

Notably, among patients <10 y/o, there was a significant difference in PFS between those that received re-irradiation compared to those that did not, while a significant difference in PFS was not observed amongst patients ≥10 y/o (potentially due to being underpowered). This suggests that patients <10 y/o may be more likely to experience rapid disease progression and not be candidates for re-irradiation regardless of shared decision-making discussions.

Limitations

The most significant limitation of the present study is its retrospective nature and overall small number of patients. However, the present study remains one of the largest series of molecularly characterized tumors. DMGs are rare and molecularly characterized tumors for which robust molecular and clinical data is still sparse. Our current study presents a relatively large collection of fully characterized, previously unreported cases from a single institution that presents molecular and clinical distinctions between "adolescent" DMGs and younger "child" pediatric DMGs and opens the door for further corroboration. A potential confounder to our findings is the heterogeneity of disease management with a high degree of variability amongst the systemic therapies delivered. However, this confounder is less likely to have impacted our findings on age of onset, as there was a random distribution of primary systemic therapies across our cohort. Another potential confounder is that patients ≥10y/o had a higher proportion of debulking surgery compared to patients <10 y/o, but there was no significant effect on OS via log-rank test or Cox regression analysis, and the lack of survival benefit with increased resection for DMGs is widely accepted and was shown in a larger series.[5] An additional limitation of our study is that our search terms did not include "grade 2", which could have reduced our cohort size. While we accounted for DMG patients with low-grade histology because every patient that had a pathology report with an integrated molecular diagnosis of DMG included the terms "WHO grade 4" or "WHO grade 3/4" regardless of histologic grade, it is possible that some DMG patients with low-grade histology were not pulled by our search strategy. However, aside from having a reduced cohort size, we do not believe this would have affected our findings given that DMGs with low grade histology are well established as having the same outcomes as DMGs with high-grade histology.[2]

In conclusion, diffuse midline gliomas are rare and deadly brain tumors for which safe surgical resection is commonly not an option. Despite numerous attempts at novel treatment, survival for DMGs has not improved in the last decade. Increased understanding of prognostic factors like patient age at onset and molecular characterization will lead to better and risk-adapted treatment strategies. We identified age ≥10 y/o, or "adolescent DMGs," having improved survival compared to younger "child" pediatric DMGs (age <10 y/o) in our cohort. We additionally identified high rates of *PI3K/AKT* pathway alterations in DMGs <10 y/o only. "Adolescent" DMGs having clinical and molecular distinctions from earlier-onset pediatric DMGs has implications in clinical trial design and interpretation, as well as clinical management decisions such as the viability of re-irradiation, though the present study is not large enough to definitively establish that. On the re-irradiation front, we provide the first report of re-irradiation in an entirely molecularly diagnosed DMG cohort and support that re-irradiation is a relatively safe option for progressive DMGs.

Keywords

diffuse midline gliomas | K27M | adolescent | PI3K/AKT signaling | re-irradiation

Funding

The authors declare that no other funds, grants, or other support were received during the preparation of this manuscript.

Conflict of Interest Statement. Y.Y., N.W., Y.A., S.G., S.S., D.B., R.S., and M.D. declare no relevant conflict of interests. A.S. received a R35CA197569 grant as the PI for an unrelated project.

Author Contributions

Y.Y. and M.D. conceptualized the project. Y.Y. and M.D. wrote the project specific IRB protocol. Y.Y. and N.W. collected patient data and performed survival analyses. Y.Y. performed the multivariate Cox regression analysis. Y.A. and A.S. performed the gene ontology and related analyses. Y.Y., D.B., R.S., and M.D. wrote the manuscript. N.W., S.G., S.S., Y.A., and A.S. participated in the editing of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

This study was received IRB approval from Lurie Children's Hospital of Chicago, IRB #LCH2022-5620.

Affiliations

Department of Neurological Surgery, Northwestern Lou and Jean Malnati Brain Tumor Institute, Northwestern University Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois (Y.Y., S.G., M.D.); Division of Pediatric Neurosurgery, Ann and Robert Lurie Children's Hospital of Chicago, Chicago, Illinois (Y.Y., M.D.); Division of Neuro-Oncology, Northwestern Lou and Jean Malnati Brain Tumor Institute, Northwestern University Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois (Y.Y., R.S.); Department of Pathology and Laboratory Medicine, Stanley Manne Children's Research Institute, Ann and Robert Lurie Children's Hospital of Chicago, Chicago, Illinois (N.W.); Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, Illinois (Y.A., A.S.); Department of Radiation Oncology, Northwestern Lou and Jean Malnati Brain Tumor Institute, Northwestern University Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois (S.S.); Department of Pathology, Northwestern Lou and Jean Malnati Brain Tumor Institute, Northwestern University Robert H. Lurie Comprehensive Cancer Center, Chicago, Illinois (D.B.)

References

1. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23:1231-1251.
2. Gessi M, Gielen GH, Dreschmann V, Waha A, Pietsch T. High frequency of H3F3A K27M mutations characterizes pediatric and adult high-grade gliomas of the spinal cord. *Acta Neuropathol*. 2015;130:435-437.
3. Mackay A, Burford A, Carvalho D, et al. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell*. 2017;32:520-537. e525.
4. Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, et al. Clinical, radiologic, pathologic, and molecular characteristics of long-term survivors of diffuse intrinsic pontine glioma (DIPG): a collaborative report from the international and European Society for Pediatric Oncology DIPG Registries. *J Clin Oncol*. 2018;36:1963-1972.
5. Karremann M, Gielen GH, Hoffmann M, et al. Diffuse high-grade gliomas with H3 K27M mutations carry a dismal prognosis independent of tumor location. *Neuro Oncol*. 2018;20:123-131.
6. Solomon DA, Wood MD, Tihan T, et al. Diffuse midline gliomas with histone H3-K27M mutation: a series of 47 cases assessing the spectrum of morphologic variation and associated genetic alterations. *Brain Pathol*. 2016;26:569-580.
7. Ryall S, Krishnatry R, Arnoldo A, et al. Targeted detection of genetic alterations reveal the prognostic impact of H3K27M and MAPK pathway aberrations in paediatric thalamic glioma. *Acta Neuropathol Commun*. 2016;4:93.
8. Dorfer C, Czech T, Gojo J, et al. Infiltrative gliomas of the thalamus in children: the role of surgery in the era of H3 K27M mutant midline gliomas. *Acta Neurochir (Wien)*. 2021;163:2025-2035.
9. Cooney T, Lane A, Bartels U, et al. Contemporary survival endpoints: an international diffuse intrinsic pontine glioma registry study. *Neuro Oncol*. 2017;19:1279-1280.
10. Wolff JE, Rytting ME, Vats TS, et al. Treatment of recurrent diffuse intrinsic pontine glioma: the MD Anderson Cancer Center experience. *J Neurooncol*. 2012;106:391-397.
11. Vanan MI, Eisenstat DD. DIPG in children–what can we learn from the past? *Front Oncol*. 2015;5:237.
12. Janssens GO, Gandola L, Bolle S, et al. Survival benefit for patients with diffuse intrinsic pontine glioma (DIPG) undergoing re-irradiation at first progression: a matched-cohort analysis on behalf of the SIOP-E-HGG/DIPG working group. *Eur J Cancer*. 2017;73:38-47.
13. Buczkowicz P, Hawkins C. Pathology, molecular genetics, and epigenetics of diffuse intrinsic pontine glioma. *Front Oncol*. 2015;5:147.
14. Schwartzentruber J, Korshunov A, Liu X-Y, et al. Driver mutations in histone H3. 3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012;482:226-231.
15. Castel D, Kergrohen T, Tauziède-Espariat A, et al. Histone H3 wild-type DIPG/DMG overexpressing EZHIP extend the spectrum diffuse midline gliomas with PRC2 inhibition beyond H3-K27M mutation. *Acta Neuropathol*. 2020;139:1109-1113.
16. Khuong-Quang D-A, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3. 3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol*. 2012;124:439-447.
17. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet*. 2014;46:451-456.
18. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat Genet*. 2014;46:462-466.
19. Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet*. 2014;46:444-450.
20. Castel D, Philippe C, Calmon R, et al. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol*. 2015;130:815-827.
21. Bender S, Tang Y, Lindroth AM, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell*. 2013;24:660-672.
22. Lewis PW, Müller MM, Koletsky MS, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science*. 2013;340:857-861.
23. Sievers P, Sill M, Schrimpf D, et al. A subset of pediatric-type thalamic gliomas share a distinct DNA methylation profile, H3K27me3 loss and frequent alteration of EGFR. *Neuro Oncol*. 2021;23:34-43.
24. Chan KM, Fang D, Gan H, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev*. 2013;27:985-990.
25. Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell*. 2012;22:425-437.
26. Taylor KR, Mackay A, Truffaux N, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nat Genet*. 2014;46:457-461.
27. Zheng L, Gong J, Yu T, et al. Diffuse midline gliomas with histone H3 K27M mutation in adults and children: a retrospective series of 164 cases. *Am J Surg Pathol*. 2022;46:863-871.
28. Stegat L, Eckhardt A, Gocke A, et al. Integrated analyses reveal two molecularly and clinically distinct subtypes of H3 K27M-mutant diffuse midline gliomas with prognostic significance. *Acta Neuropathol*. 2024;148:40.
29. Schulte JD, Buerki RA, Lapointe S, et al. Clinical, radiologic, and genetic characteristics of histone H3 K27M-mutant diffuse midline gliomas in adults. *Neuro-Oncol Adv*. 2020;2:vdaa142.
30. Cheng L, Wang L, Yao Q, et al. Clinicoradiological characteristics of primary spinal cord H3 K27M-mutant diffuse midline glioma. *J Neurosurg Spine*. 2022;36:303-314.

31. Yao J, Wang L, Ge H, Yin H, Piao Y. Diffuse midline glioma with H3 K27M mutation of the spinal cord: a series of 33 cases. *Neuropathology*. 2021;41:183-190.
32. Roberts HJ, Ji S, Picca A, et al. Clinical, genomic, and epigenomic analyses of H3K27M-mutant diffuse midline glioma long-term survivors reveal a distinct group of tumors with MAPK pathway alterations. *Acta Neuropathol*. 2023;146:849-852.
33. Schüller U, Iglauer P, Dorostkar MM, et al. Mutations within FGFR1 are associated with superior outcome in a series of 83 diffuse midline gliomas with H3F3A K27M mutations. *Acta Neuropathol*. 2021; 141:323-325.
34. Dono A, Takayasu T, Ballester LY, Esquenazi Y. Adult diffuse midline gliomas: clinical, radiological, and genetic characteristics. *J Clin Neurosci*. 2020;82:1-8.
35. Lapin DH, Tsoli M, Ziegler DS. Genomic insights into diffuse intrinsic pontine glioma. *Front Oncol*. 2017;7:57.
36. Hamisch C, Kickingereder P, Fischer M, Simon T, Ruge MI. Update on the diagnostic value and safety of stereotactic biopsy for pediatric brainstem tumors: a systematic review and meta-analysis of 735 cases. *J Neurosurg Pediatr*. 2017;20:261-268.
37. Li D, Bonner ER, Wierzbicki K, et al. Standardization of the liquid biopsy for pediatric diffuse midline glioma using ddPCR. *Sci Rep*. 2021;11:5098.
38. Liu I, Jiang L, Samuelsson ER, et al. The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location. *Nat Genet*. 2022;54:1881-1894.
39. Cox JD, Stetz J, Pajak TF. Toxicity criteria of the radiation therapy oncology group (RTOG) and the European organization for research and treatment of cancer (EORTC). *Int J Radiat Oncol Biol Phys*. 1995;31:1341-1346.
40. Yu G, He Q-Y. ReactomePA: an R/Bioconductor package for reactome pathway analysis and visualization. *Mol Biosyst*. 2016;12:477-479.
41. Korshunov A, Ryzhova M, Hovestadt V, et al. Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers. *Acta Neuropathol*. 2015;129:669-678.
42. Jain SU, Do TJ, Lund PJ, et al. PFA ependymoma-associated protein EZHIP inhibits PRC2 activity through a H3 K27M-like mechanism. *Nat Commun*. 2019;10:2146.
43. Mondal G, Lee JC, Ravindranathan A, et al. Pediatric bithalamic gliomas have a distinct epigenetic signature and frequent EGFR exon 20 insertions resulting in potential sensitivity to targeted kinase inhibition. *Acta Neuropathol*. 2020;139:1071-1088.
44. Arafeh R, Samuels Y. PIK3CA in cancer: the past 30 years. *Semin Cancer Biol*. 2019;59:36-49.
45. Filbin M, Monje M. Developmental origins and emerging therapeutic opportunities for childhood cancer. *Nat Med*. 2019;25:367-376.
46. Kline C, Liu SJ, Duriseti S, et al. Reirradiation and PD-1 inhibition with nivolumab for the treatment of recurrent diffuse intrinsic pontine glioma: a single-institution experience. *J Neurooncol*. 2018;140:629-638.
47. Lassaletta A, Strother D, Laperriere N, et al. Reirradiation in patients with diffuse intrinsic pontine gliomas: the Canadian experience. *Pediatr Blood Cancer* 2018;65:e26988.