LETTER TO THE EDITOR

Brain
Pathology

IDH-mutant brainstem gliomas in adolescent and young adult patients: Report of three cases and review of the literature

In contrast to adult gliomas, isocitrate dehydrogenase (IDH) mutations are rare in pediatric and adolescent gliomas. In a recent series of over 1000 pediatric low grade gliomas (LGG), the *IDH1* R132H mutation was found in only 0.8% of studied cases, with those identified occurring in older children/adolescents (median 15.7 years) and exclusively in the cerebral hemispheres (1). Other studies show a slightly higher incidence (up to 16%) of IDH mutations in pediatric high grade gliomas (HGG), with comparable age range and exclusivity to hemispheric locations (2).

IDH-mutant gliomas arising in the brainstem are exceedingly rare in both the adult and pediatric population. Infratentorial location is uncommon in diffuse gliomas, with 5% or less occurring in the brainstem, cerebellum, or spinal cord, and IDH mutations in the infratentorial compartment appear to be rare (7%) (3–5). Brainstem gliomas are very rare in adults, but approximately 10% of pediatric CNS tumors occur in this location, with diffuse midline glioma, K27M-mutant representing most cases.

We recently identified three adolescent/young adult (AYA) patients (Figure 1A) with IDH-mutant glioma of the brainstem with several key clinicopathologic and molecular features in common (summarized in Table S1). Patient #1 was a 19-year-old female who presented with left ptosis, left facial palsy, headache, diplopia, ataxia, and dysarthria. Magnetic resonance imaging (MRI) of the brain revealed a diffusely infiltrating and enlarging space-occupying lesions within the brainstem without enhancement. She did not have a biopsy on initial presentation because of concerns for significant morbidity and was initially treated with focal intensity-modulated radiation therapy (IMRT) with concurrent temozolomide (TMZ) after magnetic resonance spectroscopy (MRS) showed findings consistent with LGG. Three years after completing radiation therapy, she developed worsening neurological symptoms and was found to have progressive disease on MRI with extension of the tumor into the facial colliculi bilaterally. MRS findings remained consistent with LGG and she was treated with carboplatin, vincristine, and TMZ per ACNS0223. After five cycles of chemotherapy (56 months from initial diagnosis), she underwent tissue sampling due to disease progression with MRI findings of new areas of infiltrative T2/FLAIR signal hyperintensity throughout the right cerebellar parenchyma. Histopathology and molecular analysis led to an integrated diagnosis of anaplastic astrocytoma, *IDH1*-mutant, WHO grade 3. Despite aggressive therapy, Patient #1 progressed on treatment and died 62 months after initial diagnosis.

Patient #2 was a 17-year-old male who presented with left leg weakness, headache, diplopia, ataxia, and left facial palsy. MRI of the brain demonstrated an expansile, diffusion-restricted lesion in the right pons with no evidence of contrast enhancement. He received up-front biopsy with histopathology and molecular analyses that were consistent with an infiltrating astrocytoma, *IDH1*-mutant, WHO grade 2. He was treated with concurrent chemoradiation using focal IMRT and TMZ. He received adjuvant cycles of TMZ and continues to have stable disease 3 years after diagnosis.

Patient #3 was a 24-year-old male who presented with right facial palsy and diplopia. Brain MRI showed an infiltrative, expansile lesion of the brainstem with small areas of enhancement (Figure 1B,C). He underwent biopsy, which led to the integrated diagnosis of infiltrating glioma, *IDH1*-mutant, WHO grade 2. Similar to Patient #2, he was treated with concurrent chemoradiation using focal IMRT and TMZ with adjuvant cycles of TMZ. He continues to have stable disease 1.5 years after diagnosis.

As shown in Figure 1D–G and Figure S1, the three cases by histology were diffuse gliomas with low to moderate Ki-67 labeling indices. Tumors from Patients #2 and #3 were positive for *IDH1* R132H by immunohistochemistry and confirmatory sequencing, while Patient #1 had a noncanonical *IDH1* R132S mutation by next-generation sequencing. The tumors of Patients #1 and #3 showed diminution or loss of immunoexpression of H3K27me3. None of the three tumors demonstrated loss of ATRX immunoreactivity or *ATRX* mutation. Pathogenic variants of *TP53* were identified in all three cases. Other genetic alterations identified included *MET* exon 14 skipping (Patient #1) and *MYCN* amplification

(A)

| Patient	Histology	Age	Sex	Tumor Location	Clinical presentation	IDH1 variant	ATRX variant	TP53 variant	Other genetic alterations
1	Anaplastic astrocytoma	19	F	Brainstem	Left-sided ptosis, facial palsy	R132S	no	p.R273H	*MET* exon 14 skipping
2	Diffuse astrocytoma	17	M	Brainstem	Ataxia, diplopia, left facial palsy	R132H	no	p.R248W, p.E286K	*MYCN* amplification
3	Infiltrating glioma	24	M	Brainstem	Right facial palsy, diplopia	R132H	no	p.V173A, p.V157_R158del	None
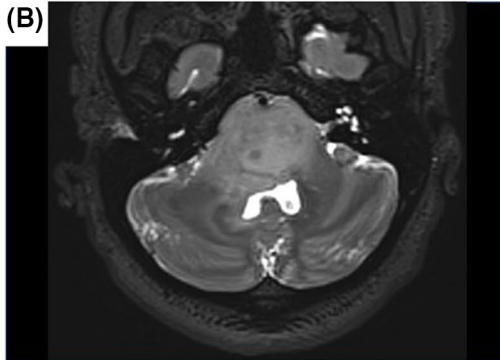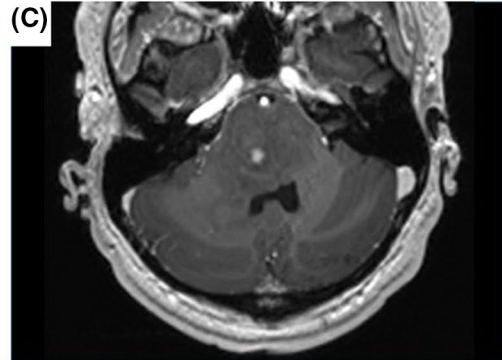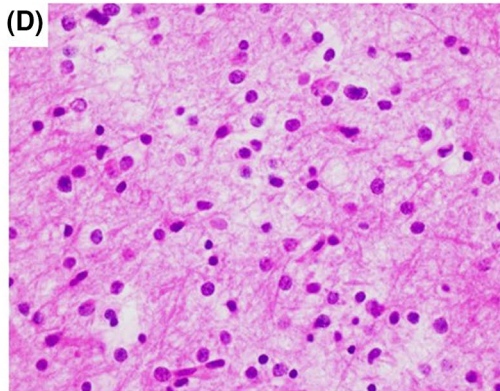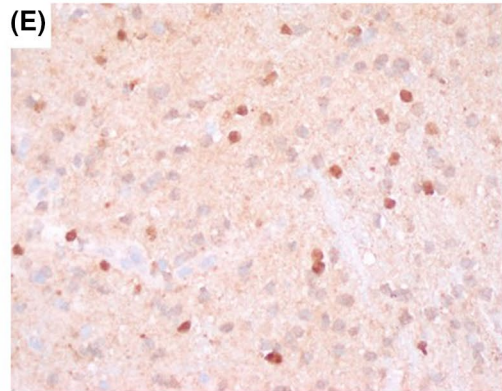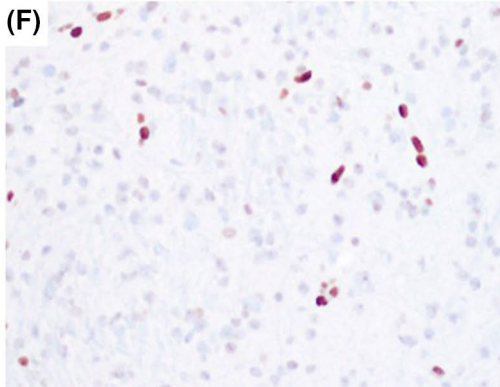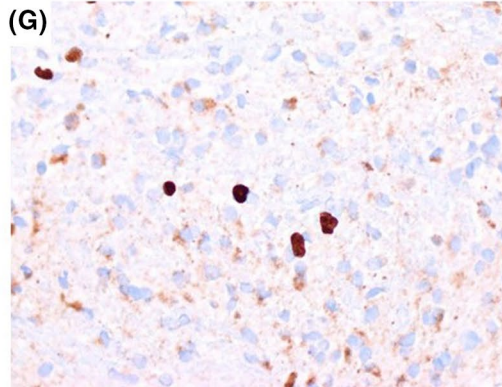
FIGURE 1 IDH-mutant brainstem gliomas in adolescent/young adult patients. (A) Clinicopathologic features of the three patients with IDH-mutant brainstem gliomas and associated genetic alterations identified in tumor tissue. (B and C) Preoperative axial MRI, (B) T2-weighted, and (C) T1-weighted post-contrast of Patient 3. (D–G) Histologic and immunohistochemical features of tumor from Patient #3. (D) H&E staining, (E) IDH R132H immunostaining, (F) H3K27me3 immunostaining, and (G) Ki-67 immunostaining

(Patient #2) (gene content for next-generation sequencing panels is provided in Tables S3 and S4). None of the tumors showed evidence of 1p/19q codeletion.

When compared with pediatric LGG, adult LGG more frequently harbor IDH mutations and demonstrate poorer long-term survival with a higher risk of malignant transformation and death (6). The relative survival advantage conferred by IDH mutation in other anatomic locations is not observed in the brainstem, with overall survival similar to supratentorial IDH wild-type gliomas (7). Generally, pediatric LGG patients have excellent long-term overall survival with rare malignant

transformation, leading to the practice of monitoring select patients with expectant observation to prevent treatment-associated morbidity (8). However, compared to other pediatric LGG patients, young people with IDH-mutant LGG appear to have a higher risk for malignant transformation, similar to their adult counterparts. For this reason, pediatric IDH-mutant LGG has been considered "intermediate risk" by some groups (1).

IDH-mutant gliomas of the infratentorial compartment may be an under-recognized category of brain tumors due to historical reluctance to biopsy this location and presumption of tumor grade based on MRI features. In a recent study of brainstem gliomas, IDH-mutant tumors composed a small but distinct subset (9), with a median age of 39, and the most frequent histologic patterns being WHO grade 2 (66.7%) or WHO grade 3 (26.7%). DNA methylation studies and copy number profiling suggest that IDH-mutant infratentorial gliomas are molecularly distinct and may arise from a different cell of origin than their supratentorial counterparts (7). The same study also showed that infratentorial IDH-mutant astrocytomas have a predominance of noncanonical IDH variants and a low rate of ATRX loss compared to supratentorial tumors. Outcome analysis showed overall survival in patients with infratentorial IDH-mutant astrocytomas to be longer than those with diffuse midline gliomas, but shorter than patients with supratentorial IDH-mutant astrocytomas, specifically those classified as "low grade" by methylation subclass. The existing literature on IDH-mutant brainstem gliomas in pediatric and AYA patients is summarized in Table S2.

Recognition of this category of tumors has practical implications for neuropathology practice and requires special attention to immunohistochemical workup. Two of three cases in our series showed partial or complete loss of H3K27me3 protein expression (Figure S1). In the context of brainstem glioma, this pattern is typically considered supportive or diagnostic of diffuse midline glioma. These cases demonstrate that caution should be taken in interpreting this stain without confirmation of a diffuse midline glioma-associated alteration (e.g., H3 K27M or *EGFR* mutation, EZHIP overexpression). *ATRX* mutations and thus ATRX loss were not observed in this group, in contrast to the typical pattern for supratentorial IDH-mutant astrocytomas, suggesting ATRX immunohistochemistry may be less useful in these cases. Finally, there is evidence that noncanonical *IDH1* mutations are enriched in IDH-mutant infratentorial tumors, making *IDH1* immunohistochemistry of more limited utility (as demonstrated by Patient #1) (5, 7, 9). Still, Patients #2 and #3 illustrate the importance of *IDH1* R132H immunohistochemistry in the setting of AYA brainstem glioma.

While not present in the cases we identified, two brainstem gliomas with co-occurring IDH and H3 K27M mutations have been recently reported ((7), Table S2). Previously, IDH and H3 K27M mutations had been considered mutually exclusive. Both of the reported cases with co-occurring *IDH1* and H3 K27M mutations harbored noncanonical *IDH1* mutations (R132C), underscoring the importance of sequencing-based assays for these tumors. Both tumors had short overall survival and methylation profiles generally aligned with other IDH-mutant infratentorial gliomas.

Without biopsy, there is currently no method to distinguish IDH-mutant brainstem gliomas from other LGG or HGG entities with a wide range of prognoses. Of note, *MET* exon 14 skipping or *MYCN* amplification, which were identified in our patients' tumors, are uncommon findings in LGG, and *MYCN* amplification is associated with poor prognosis in IDH-mutant gliomas (10). This series highlights that, while uncommon, IDH-mutant tumors occur in the brainstem of AYA patients, can show low grade histologic features, and should be identified by up-front immunohistochemical and molecular testing. In our patients, the detection of IDH mutations did not directly impact treatment decisions due to the patients' severe symptomatology requiring urgent radiation therapy. However, in patients for whom avoidance of radiation therapy is feasible, the presence of IDH mutations could lead to a chemotherapy-only approach given the tumors' potential to undergo malignant transformation. In the near future, knowledge of the presence of IDH mutations will enable consideration of emerging therapies. Biopsy is critical for patient selection in the recurrent setting, for example, imipridone (ONC201) in H3 K27M-mutant gliomas and ongoing investigations with IDH inhibitors and vaccines in IDH-mutant LGG. Additionally, preclinical studies have shown that IDH mutations may be predictive of response to PARP inhibitors through synthetic lethality, which is currently being evaluated (NCT03212274). Overall, these findings underscore the importance of performing biopsies of brainstem tumors to establish a specific diagnosis, more accurate prognostication, and appropriate treatment.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 Histologic features of tumors from Patients #1-3. Biopsy of recurrent tumor from Patient #1 (top row) showed tumor cells infiltrating the cerebellar white matter. *IDH1* R132H immunohistochemistry was negative, concordant with the observed R132S mutation in this patient. H3K27me3 showed diminished nuclear immunoexpression with a subset of tumor cells appearing negative. Ki-67 labeling index was moderate to high. The overall features were consistent with an anaplastic astrocytoma, WHO grade 3. Biopsy from the brainstem of Patient #2 (middle row) showed increased cellularity with an infiltrative growth pattern of tumor cells marked by irregular nuclear contours. The findings were consistent with an infiltrative astrocytoma, WHO grade 2. Immunohistochemistry for *IDH1* R132H was positive in the scattered tumor cells, H3K27me3 expression was retained, and Ki-67 labeling index was low. Biopsy from Patient #3 (bottom row) showed an infiltrating glioma with mild nuclear atypia. Immunohistochemistry for *IDH1* R132H was positive in scattered cells. H3K27me3 expression was lost in tumor nuclei. Ki-67 labeling index was low to moderate

TABLE S1 Pathogenic genetic alterations identified in the three brainstem gliomas

TABLE S2 Summarized clinicopathologic and genetic data from Patients #1-3 and review of the literature. Includes reported grade 2-4 IDH-mutant tumors arising in the brainstem in patients under age 30

TABLE S3 List of targeted genes in CHLA OncoKids Cancer Panel

TABLE S4 List of targeted genes in Johns Hopkins Molecular Diagnostics Laboratory Solid Tumor Panel.