Adult Brainstem Gliomas With H3K27M Mutation: 
Radiology, Pathology, and Prognosis

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Abstract
Adult brainstem gliomas are difficult to classify based on radiologic and histologic features. A K27M mutation in histone 3 has been described to identify high-grade midline gliomas associated with a particularly unfavorable prognosis. While initially considered a pediatric entity, it is now known that H3K27M-mutant brainstem gliomas occur in all age groups, but they are less well understood in adults. We studied clinical, radiologic, and pathologic features of 25 brainstem gliomas diagnosed at our institution between 1994 and 2017 in subjects at least 18 years old. Seven tumors (28%) were positive for the H3K27M mutation, and their median overall survival was significantly shorter than in the H3-wildtype group (p = 0.004). Although the mutation was invariably associated with a poor prognosis, our study also illustrates the radiologic and pathologic heterogeneity in this molecular tumor subtype. The results showed that H3K27M-mutant status and clinically aggressive course cannot be ruled out based on low-grade histology on the initial biopsy, exophytic growth, only focal or minimal enhancement or an extrapontine location, such as midbrain or medulla. These results favor an integrated approach employing a combination of clinical, radiologic, histologic features as well as H3K27M immunohistochemistry for the diagnostic subclassification of adult brainstem gliomas.

Key Words: Adult, Brainstem, Exophytic, Glioma, H3K27M mutation, Midline, Survival.

INTRODUCTION
Adult brainstem gliomas are a subtype of diffuse midline gliomas that are not well understood or characterized. They arise in the midline structures of the brainstem (midbrain, pons, and medulla), cerebellar peduncles, and cerebellopontine angle. The majority of data that contribute to our understanding of these tumors come from studies of pediatric and young adult patients with high-grade gliomas. Molecular analysis of diffuse intrinsic pontine gliomas (DIPGs) in children revealed a unique recurrent lysine to methionine substitution at codon 27 (K27M) in histone H3 variants, H3.3 gene H3F3A (~75%) and less frequently in H3.1 gene HIST1H3B (~25%) in ~80% of high-grade DIPGs (1–4). In addition to DIPGs, it was also present in most other pediatric infiltrative astrocytomas arising in midline structures (1, 3, 5–8). The mutation was originally believed to be limited to pediatric gliomas; however, more recent data demonstrate that it also occurs in young and older adults (5, 6, 9, 10), and can also arise in the cerebellum and other nonmidline sites (11). The prevalence of K27M mutation is significantly higher in adult brainstem gliomas compared to supratentorial gliomas (12). It has been shown in pediatric studies that H3K27M-mutant gliomas exhibit aggressive clinical behavior and carry a poor prognosis, irrespective of the histologic features (2–4, 8, 10, 13), which prompted their classification as a separate entity in the World Health Organization (WHO) Classification of Tumours of the Central Nervous System 2016 (14, 15). The nature of H3K27M gain-of-function mutation is such that it alters gene expression by posttranslational H3 modifications leading to altered DNA methylation, which collectively drive gliomagenesis via epigenetic regulation (16–18). More specifically, H3K27M sequesters PRC2 histone methyltransferase, suppressing its function and drastically decreasing the levels of wildtype trimethylated H3K27 (H3K27me3), which normally serves as a transcriptional repressor. This significant reduction in H3K27me3 is thought to result in an extensive transcriptional reprogramming of the tumor cells by increasing the expression of cancer-related genes (18–20). There are multiple other gene alterations that have been shown to associate with histone gene mutations. Tumor suppressor p53 mutations are found in half of pediatric gliomas and overlap significantly with H3F3A mutations (4, 21). Mutations in the histone
chaperone alpha-thalassemia/mental retardation syndrome X-linked (ATRX) affect the loading of histone H3.3 in heterochromatic regions of telomeres (22), and combined H3F3A mutations and ATRX mutations are strongly associated with telomerase-independent lengthening of telomeres (21). An ATRX mutation is more common in older children and adolescents, and has a predilection for thalamic and spinal regions (10). ATRX loss in adults frequently co-occurs with H3 mutations, and itself is a useful biomarker in the refinement of the diagnosis of IDH-mutant astrocytomas (13).

Studies have shown that brainstem gliomas are generally less aggressive in adults than in children (23, 24). However, it still remains a subject of debate whether H3K27M-mutant adult tumors exhibit a similar biology and behavior as their pediatric counterparts. Some recent studies that set out to elucidate the clinicopathologic features of mixed pediatric and adult midline gliomas and other entities such as gangliogliomas with H3K27M mutation show a trend for them to follow a similar clinical course, at least in midline structures other than the thalamus (10, 25). Moreover, other studies showed no difference in the prognosis of adult patients with H3K27M-mutated thalamic gliomas compared to their H3-wildtype counterparts, and a better prognosis for H3-mutant thalamic gliomas compared to H3-mutant brainstem gliomas (5, 6). Another recent study of 21 midline H3K27M-mutant gliomas including thalamus showed no difference in the survival compared with H3-wildtype tumors (26). Case reports of H3-positive pilocytic astrocytomas in a 7-year-old child, a young adult and a 53-year-old patient, as well as a glioneuronal tumor in a 10-year-old child with less aggressive phenotypes have also been published (9, 27, 28). These observations collectively suggest that some form of heterogeneity is occurring in this molecular tumor subtype, possibly dependent on the biology specific to each anatomic location, and also within each age category. Because the presence of this mutation may predict an aggressive clinical course while simultaneously providing an opportunity for new targeted therapies, such as panobinostat and JMJD3 (20, 29, 30), there is an increasing need to identify these molecular aberrations. The purpose of the present study was to characterize adult brainstem gliomas, with a focus on the H3K27M mutation status, radiologic features, histologic features, and clinical outcome.

MATERIALS AND METHODS

Patient Inclusion Criteria

Patients were included in this IRB-approved, retrospective case series if they met the following criteria: 1) They were at least 18 years of age at the time of initial presentation, 2) they had a histologic diagnosis of glioma, and 3) tumor was centered in the midbrain, pons, medulla, cerebellar peduncle, or cerebellopontine angle.

Data Collection

A total of 25 adult patients meeting the inclusion criteria were identified in our institution’s neuropathology files between 1994 and 2017. The following data were obtained from the electronic medical record and from the UT Southwestern Cancer Center Tumor Registry: 1) age at the time of diagnosis, 2) nature and duration of symptoms, 3) gender, 4) tumor location, 5) histologic diagnosis, 6) type of treatment, 7) length of follow-up, and 8) status at the time of last follow-up. In addition, all relevant imaging studies were reviewed, and the following data were recorded: 1) tumor location, 2) tumor size (where applicable, dimensions of both abnormal FLAIR signal and enhancing component were recorded), 3) signal characteristics, including presence and pattern of enhancement, 4) presence of exophytic tumor growth, and 5) presence of hydrocephalus.

Statistical Analysis

We used the Kaplan-Meier method to plot patient survival over time from the point of diagnosis and apply log-rank test using GraphPad Prism version 7.03 software (GraphPad, La Jolla, CA). For establishing the presence or absence of correlation, nonparametric Spearman correlation was used.

Microscopy

Photomicrographs were taken with a Nikon DS-Fi1-L2 digital camera on Nikon microscope (Nikon Corporation, Melville, NY) using ACT-1 software Version 2.63 (Nikon).

Cell Count

MIB-1/Ki-67 proliferation index was determined by manual morphometry using the Glasgow cell counting graticule (31).

Immunohistochemistry

Four-micrometer-thick sections of formalin-fixed, paraffin-embedded tissue underwent heat-induced epitope retrieval using CC1 (Ventana, Tucson, AZ), a Tris-based buffer at pH 8–8.5, followed by immunohistochemical (IHC) staining with a polyclonal rabbit antibody to H3K27M-mutant protein ABE419 (EMD Millipore, Billerica, MA) diluted 1:500, a monoclonal mouse antibody to Ki-67/MIB-1 (Dako, Carpinteria, CA) diluted 1:50, polyclonal rabbit antibody to ATRX (Sigma-Aldrich, St. Louis, MO) diluted 1:200, monoclonal mouse antibody to p53 (clone DO-7, Ventana) diluted 1:100, monoclonal mouse antibody to IDH1 R132H (Dianova, Hamburg, Germany) diluted 1:40, and monoclonal mouse antibody to BRAF V600E-mutant protein (VE1) (Ventana) prediluted by the manufacturer. IHC was performed on either the Dako Omnis (for H3K27M), Ventana Benchmark XT or Ventana Benchmark Ultra automated stainer, using a Ventana UltraView Universal DAB Detection Kit or OptiView DAB IHC Detection Kit, the latter for the VE1 stain only.

RESULTS

Among the 25 brainstem gliomas studied, the majority were centered in the midbrain (44%) and pons (28%), whereas the rest were located in the medulla (12%), cerebellar peduncles/pons (12%), and cerebellopontine angle (4%) (Table 1). The histologic grade distribution was almost equal, with 13 low-grade (pilocytic or diffuse astrocytoma) and 12...
high-grade (anaplastic astrocytoma or glioblastoma) cases. As expected, the survival of patients with histologically low-grade tumors was significantly greater than of those with high-grade tumors (p = 0.001, log-rank test, Fig. 1A). The majority of tumors (n = 17) had contrast enhancement, whereas 6 did not enhance, and the data on remaining 2 are not available. There was no correlation between the presence of contrast enhancement radiologically and tumor grade (p = 0.13, r = 0.32), nor was there a survival advantage for the nonenhancing tumors compared to those that enhanced (p = 0.11, log-rank test). The median age at diagnosis was 39 years (range 22–67) and there were 17 male patients and 8 female patients. We then studied if the tumor behavior was more benign when it appeared to be dorsally exophytic on imaging, as has been previously shown in the pediatric population (2, 32, 33). Even though the survival of adult patients with dorsally exophytic tumors as a group was not statistically different from those with an intrinsic component expanding the brainstem (p = 0.06, log-rank test, Fig. 1B), there was a trend toward a better survival for exophytic tumors. Twenty-three of the tumors had sufficient tissue available for a full panel of IHC stains, and a partial panel was completed on the remaining cases. All of the tested tumors were negative for IDH1-R132H and BRAF V600E mutations, whereas other stains were variably positive (Supplementary Data Tables S1 and S2).

Characteristics of Diffuse Brainstem Gliomas Without H3K27M Mutation

Eighteen of 25 (72%) studied cases were H3K27M-wildtype gliomas by IHC, which comprised a group of 11 low-grade and 7 high-grade tumors. The median age at diagnosis was 37 years (range 22–67). Eleven tumors were contrast enhancing on imaging, whereas 6 were nonenhancing, and data on one case were not reported. Three out of 14 tested cases had loss of ATRX, and 2 out of 16 tested cases had a p53 mutation (Supplementary Data Tables S1 and S2)—and these 2 aberrations did not overlap in any patient. MIB-1 index was high in only 6 cases, all of which were high-grade tumors, and low in the remaining high- and low-grade cases.

Characteristics of Diffuse Brainstem Gliomas With H3K27M Mutation

Seven out of 25 (28%) examined cases were positive for H3K27M mutation by IHC. Samples were counted positive for H3K27M if tumor cells showed diffuse and crisp nuclear staining. Nonspecific cytoplasmic granular staining was seen in macrophages, microglia, and other inflammatory cells, which was deemed H3K27M negative. Brain imaging by MRI detected contrast enhancement in all H3-positive cases including those diagnosed as low-grade, except for one glioblastoma for which MRI data were not available; however, given the histologic diagnosis of glioblastoma, the likelihood of this lesion having contrast enhancement is high. There was no correlation between the presence of contrast enhancement radiographically and H3K27M mutational status (p = 0.1, r = 0.35). The anatomic distribution of H3-mutated gliomas was as follows: midbrain (28.6%), pons (28.6%), medulla (14.3%), and cerebellar peduncle/pons (28.6%) (Table 1).

**TABLE 1.** Anatomical Distribution of All Brainstem Gliomas (n = 25) and H3K27M-Mutant Gliomas (n = 7)

<table>
<thead>
<tr>
<th>Anatomical Location</th>
<th>All Glioma Cases (% Total Brainstem Cases)</th>
<th>H3K27M-Mutant Gliomas (% Mutated Cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midbrain tegmentum</td>
<td>2 (8)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Medulla</td>
<td>3 (12)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Cerebellar peduncle</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>7</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Kaplan-Meier curves describing overall survival of midline glioma patients by grade (A), by exophytic growth properties (B), and by the H3K27M mutation status (C). (A) Overall survival by grade was significantly different, with high-grade cases averaging 9 months, whereas the overall survival for low-grade cases was not reached (p = 0.001). (B) Overall survival by exophytic growth properties was not significantly different, with intrinsic tumors averaging 22.8 months, whereas exophytic tumors survival was not reached (p = 0.06). (C) Overall survival by H3K27M mutation status was significantly different, with H3-mutant cases averaging 9 months, whereas H3-wildtype cases overall survival was not reached (p = 0.0004, log-rank test). Censored (living) patients are indicated on the graphs with tick marks.
<table>
<thead>
<tr>
<th>No.</th>
<th>Age (Years)</th>
<th>Symptoms</th>
<th>Sex</th>
<th>Anatomical Tumor Site</th>
<th>Grade</th>
<th>Treatment</th>
<th>Survival (Months)</th>
<th>Tumor Size (mm)</th>
<th>MRI</th>
<th>Exophytic Hydrocephalus</th>
<th>MIB-1 index(%)</th>
<th>IDH1-R132H</th>
<th>ATRX loss</th>
<th>P53+</th>
<th>BRAF V600E+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>Diplopia, paresis</td>
<td>M</td>
<td>Midbrain tegmentum</td>
<td>High</td>
<td>Biopsy, shunt, chemoradiation</td>
<td>22.8</td>
<td>20 × 15</td>
<td>Heterogeneous enhancement</td>
<td>–</td>
<td>+</td>
<td>34</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>Headache</td>
<td>M</td>
<td>Midbrain tectum</td>
<td>Low</td>
<td>Ventriclestomy, biopsy</td>
<td>6.0</td>
<td>32 × 28 × 27</td>
<td>Heterogeneous enhancement</td>
<td>+</td>
<td>+</td>
<td>1.9</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>Dizziness, headache, nausea, vomiting</td>
<td>F</td>
<td>Medulla</td>
<td>Low</td>
<td>Biopsy, chemoradiation, shunt</td>
<td>15.0</td>
<td>16 × 14</td>
<td>Minimal amorphous enhancement</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Gait instability, swallowing difficulties</td>
<td>M</td>
<td>Pons</td>
<td>High</td>
<td>Chemoradiation</td>
<td>9.0</td>
<td>n/a</td>
<td>Focal enhancement</td>
<td>–</td>
<td>–</td>
<td>40</td>
<td>n/a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>Bell’s palsy, dizziness, nausea, vomiting, vertigo, weakness</td>
<td>M</td>
<td>Pons</td>
<td>High</td>
<td>Chemoradiation</td>
<td>1.0</td>
<td>26 × 32</td>
<td>Poor irregular enhancement</td>
<td>–</td>
<td>–</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Hoarseness, dysphagia, headache, gait instability</td>
<td>M</td>
<td>Cerebellar peduncle/pons</td>
<td>High</td>
<td>n/a</td>
<td>10.5</td>
<td>25 × 30</td>
<td>Enhancing</td>
<td>–</td>
<td>n/a</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>n/a</td>
<td>M</td>
<td>Cerebellar peduncle/pons</td>
<td>High</td>
<td>n/a</td>
<td>6.5</td>
<td>n/a</td>
<td>n/a</td>
<td>–</td>
<td>n/a</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Cases 1–3 correspond to the instructive case descriptions. Fields marked by “n/a” indicate that data are not available.
The pathologic spectrum of H3K27M-mutant gliomas involved both low-grade and high-grade tumors at various locations (Table 2). The median age at diagnosis was 41 years, (range 25–54) with the majority of the affected patients being male (86%). Histologically, 5 of the 7 H3-mutant tumors were high-grade (71%) whereas 2 were low-grade astrocytomas (29%, instructive cases 2–3). One tumor in the tegmentum of a 41-year-old man was positive for p53 mutation, and none had a loss of ATRX. MIB-1 index was high only in high-grade tumors, and was low in 1 high-grade tumor and 2 low-grade tumors. We further highlight 3 interesting cases to illustrate the heterogeneity of these tumor presentations and molecular characteristics.

Patient 1 was a 41-year-old man who presented with 6 months of gradually worsening diplopia, numbness in right face and hand, and was diagnosed with glioblastoma (Fig. 2B). The mass was heterogeneously enhancing, expansile but nonexophytic, centered in the midbrain tegmentum with involvement of tectum, and measured 20 × 15 mm (Fig. 2A), causing hydrocephalus. MIB-1 index was high only in high-grade tumors, and was low in 1 high-grade tumor and 2 low-grade tumors. We further highlight 3 interesting cases to illustrate the heterogeneity of these tumor presentations and molecular characteristics.

Patient 2 was a 26-year-old man who presented from a foreign country with several weeks of headaches and was diagnosed with a low-grade astrocytoma on stereotactic biopsy (Fig. 3B). The exophytic tectal mass measured 32 × 28 × 27 mm, causing hydrocephalus (Fig. 3A). Postcontrast imaging showed heterogeneous enhancement. The patient underwent an endoscopic third ventriculostomy to relieve the obstruction. The MIB-1 index was low (1.9%), and retrospective H3K27M immunostain was unexpectedly strongly positive (Fig. 3C, D). It is unknown if the patient received any chemoradiation treatment, and his overall survival from the time of resection was 6 months.

Patient 3 was a 54-year-old woman who presented with 6 months of episodic dizziness, headache, nausea and vomiting, and was evaluated by imaging only, declining biopsy. Initial MRI showed an ill-defined 16 × 14 mm T2/FLAIR hyperintense dorsally exophytic medullary lesion (Fig. 4A) with minimal amorphous enhancement and no hydrocephalus (Fig. 4B). There was concurrently a separate T2/FLAIR hyperintense cortical/subcortical lesion in the left anterior temporal lobe (Fig. 4C). This patient was treated symptomatically with dexamethasone, but 14 months later her symptoms progressed and the medullary mass further radiographically enlarged to 18 × 15 mm with a significant increase in enhancing component. She finally underwent a biopsy which showed...
a predominantly noninfiltrative low-grade astrocytoma with only scattered axons (arrows) traversing tumor (Fig. 5A, C). MIB-1 proliferation index was difficult to interpret due to infiltrating microglia (Fig. 5B, D). She immediately received a shunt and 3 months after the diagnostic biopsy began radiation therapy. Five months after the biopsy and 1 month after radiation therapy completion, MRI showed enlargement of the T2/FLAIR hyperintense medullary lesion to 27 × 21 mm, with increased enhancement now measuring 21 × 26 × 25 mm (Fig. 4D). The T2/FLAIR-hyperintense left temporal lobe lesion, which had not been irradiated, also showed interval enlargement and new enhancement measuring 30 × 23 × 25 mm (Fig. 4E). A subsequent biopsy of the temporal mass 5 months later showed glioblastoma with a high MIB-1 index (Fig. 5E–G). The patient was given temozolomide chemotherapy but passed away 5 months later, unable to complete it. Retrospectively, tumor was positive for H3K27M (Fig. 5H), and ultimately had clinical and radiographic progression to glioblastoma. Overall survival from the time of initial biopsy was 15 months.

All of the 7 patients with H3-mutant tumors are deceased. The median survival in the H3K27M-positive group was 9 months (1–23 months), whereas the median survival in the H3-wildtype group was not reached (p = 0.0004; log-rank test; more than 50% of the patients were still alive in this group at the end of the follow-up period) (Fig. 1C). Finally, we compared the survival of patients with only high-grade brainstem gliomas based on H3K27M mutational status. The overall median survival between the 2 groups did not significantly differ (p = 0.28, log-rank test).

**DISCUSSION**

Adult brainstem gliomas are rather uncommon and incompletely understood, especially those with the H3K27M mutation. As most insights into these tumors were generated from studies of pediatric and young adult patients with high-grade gliomas, it is unclear whether they behave just as aggressively in adults, including those of older age. Several studies in adults have described sets of midline gliomas with H3K27M mutation; however, those were either analyzed along with pediatric cases and lack clinical follow-up and survival data, or focused on a specific anatomic site other than brainstem, or on the other hand were from a broad range of locations including thalamus, which has been shown by others to give rise to a less aggressive phenotype paralleling an increased survival (5–7, 10, 24, 26). Other interesting reports highlight patients with the H3K27M mutation, yet less aggressive clinical course (9, 27, 28). There is an indication that the effect of this mutation in different anatomical sites may be
variable, and warrants separate examination of these anatomic tumor subsets. Studies describing the histopathology of gliomas in the brainstem and complementing it with clinical follow-up data have not been published. In this study, we specifically report clinicopathologic and radiologic assessment of 25 adult brainstem gliomas, including IHC with an H3K27M-mutant specific antibody that detects mutations in histones H3.1 and H3.3 (34, 35). Although there was no difference in the mean age at diagnosis (41 years in both groups, with a median of 41 in H3K27M group and a median of 37 in H3WT group), a striking heterogeneity of anatomic sites and radiographic features and an overall aggressive clinical course in H3K27M-mutant brainstem gliomas was apparent. We further provide their additional molecular characterization by immunohistochemistry for MIB-1, IDH1-R132H, ATRX, p53, BRAF V600E, and address the existing limited understanding of these tumors in an adult patient population.

Among the 25 brainstem glioma cases, 7 (28%) were positive for the H3K27M mutation by immunohistochemistry and were composed of a diverse group of tumors. The age at diagnosis ranged between 25 and 54 years, which spans beyond the young adult age and corroborates previous observations of this mutation in older adults (26). All of the 7 patients with H3K27M-mutant tumors are deceased. Interestingly, 2 of the 7 (29%) tumors were low-grade, with a strikingly dismal survival of 6 and 15 months, comparable to glioblastomas. Remarkably, rare H3K27M-positive low-grade gliomas have previously been reported to have a better survival, in contrast to the patients in this series. The median survival of those with H3-mutant tumors was 9 months (1–23 months) and is significantly different from the H3-wildtype group.

In agreement with prior findings, the study results demonstrate that adult brainstem gliomas with H3K27M mutation are clinically aggressive, just as they are in pediatric patients, even when histologically appearing low-grade. Although the majority of the H3-positive tumors were diffuse intrinsic gliomas arising in the basal pons and cerebellar peduncles, occasional examples were centered in the midbrain and medulla and included dorsal exophytic gliomas. We observed a trend toward a longer survival for all dorsally exophytic gliomas as a group compared to those with an intrinsic component expanding the brainstem, and a larger study may demonstrate a significant difference, as has been shown in children. Here, we show that in contrast to pediatric tumors, select cases of exophytic tumors arising from the dorsal aspect of the brainstem...
stem in adults may harbor an H3K27M mutation and follow an aggressive clinical course (32, 33). Our results suggest that favorable radiologic and pathologic features (exophytic growth on imaging, low-grade histology) do not guarantee an indolent clinical course in adults like they do in children, and should be followed up by H3K27M testing. The value of the H3 mutation as a prognostic marker in gliomas that are histologically high-grade is less clear from our data, as the prognosis is uniformly poor in that group regardless of the H3K27M status. However, with a higher sample size, a difference may emerge, eliminating the need to test high-grade tumors. At this point, we recommend H3K27M immunohistochemistry for all brainstem gliomas, in order to identify cases at risk for a rapid clinical progression, as our data suggest.

FIGURE 5. Instructive Case 3: 54-year-old female with 15-month survival, histology. (A) Initial biopsy of the medullary mass showed low-grade astrocytoma (WHO grades I–II), NOS. (B) MIB-1 proliferation index was difficult to interpret due to infiltrating microglia and was estimated at 5%. (C) Immunohistochemistry (SMI-31 antibody) showed only scattered axons (arrows) traversing tumor, suggesting that the tumor was predominantly noninfiltrative. (D) The tumor contained frequent CD68-positive reactive microglia. (E) Subsequent biopsy of the separate temporal lobe lesion showed an infiltrating astrocytoma with scattered mitoses (arrow) and (F) microvascular proliferation, defining the tumor as glioblastoma. (G) MIB-1 proliferation index was high. (H) H3K27M immunostain was positive, consistent with future clinical progression to glioblastoma.
H3K27M expression has been shown to synergize with p53 loss in neural progenitor cells derived from human embryonic stem cells (36), and about half of pediatric midline gliomas are known to harbor a p53 mutation (8). A recent study of pediatric and adult gliomas showed an association between H3K27M mutation and p53 overexpression/ATRX loss in tumors located in the thalamus,pons, and spinal cord (10); however, unlike in our study, tumors centered in the midbrain or medulla were not included. Another study of adult gliomas limited to thalamus revealed an ATRX mutation in 29% of the tumors (5). ATRX loss has been reported almost exclusively in IDH-mutant tumors in adults (13). In contrast, in our adult series, the prevalence of p53 overexpression, suggestive of a p53 mutation, was much lower, with a single positive case among the 6 H3K27-mutant tumors and 2 positive cases among the 16 H3-wildtype tumors. Moreover, no ATRX loss was detected in the H3-mutant tumors and only 3 out of 14 H3 wildtype cases had ATRX loss, none of which overlapped with p53 overexpression or an IDH1-R132H mutation—in fact none of the 25 cases had an IDH1-R132H mutation. None of the cases stained positively for BRAF V600E, which is consistent with previously reported data (10). Some of these differences in the previously reported series and our data raise the possibility of a different biologic behavior depending on the age of the patient and the specific location of the tumor.

Interestingly, there was contrast enhancement in all of the H3K27M-positive cases for which radiology data were available (6/7), but enhancement was also detected in nearly half of the H3-wildtype cases, and no correlation was detected between enhancement and H3K27M mutational status. A recent study analyzing 33 diffuse midline glioma patients, including 24 with H3K27M mutation concluded on their diverse histologic cases had ATRX loss, none of which overlapped with p53 overexpression or an IDH1-R132H mutation—in fact none of the 25 cases had an IDH1-R132H mutation. None of the cases stained positively for BRAF V600E, which is consistent with previously reported data (10). Some of these differences in the previously reported series and our data raise the possibility of a different biologic behavior depending on the age of the patient and the specific location of the tumor.

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In summary, we characterized 25 adult brainstem gliomas using imaging, histology, immunohistochemistry, and clinical data. We further examined H3K27M-mutant tumors and determined that even though they were diverse histologically and radiologically, their survival was significantly decreased. We recommend that all brainstem and other midline gliomas undergo IHC testing for H3K27M mutation.

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