» This article has been updated from its originally published version to correct a term used in the article. See the corresponding erratum notice in this issue, p 844. «

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CLINICAL ARTICLE

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# A multicenter analysis of the prognostic value of histone H3 K27M mutation in adult high-grade spinal glioma

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**OBJECTIVE** High-grade spinal glioma (HGSG) is a rare but aggressive tumor that occurs in both adults and children. Histone H3 K27M mutation correlates with poor prognosis in children with diffuse midline glioma. However, the role of H3 K27M mutation in the prognosis of adults with HGSG remains unclear owing to the rarity of this mutation, conflicting reports, and the absence of multicenter studies on this topic.

**METHODS** The authors studied a cohort of 30 adult patients with diffuse HGSG who underwent histological confirmation of diagnosis, surgical intervention, and treatment between January 2000 and July 2020 at six tertiary academic centers. The primary outcome was the effect of H3 K27M mutation status on progression-free survival (PFS) and overall survival (OS).

**RESULTS** Thirty patients (18 males and 12 females) with a median (range) age of 50.5 (19–76) years were included in the analysis. Eighteen patients had H3 K27M mutation–positive tumors, and 12 had H3 K27M mutation–negative tumors. The median (interquartile range) PFS was 3 (10) months, and the median (interquartile range) OS was 9 (23) months. The factors associated with increased survival were treatment with concurrent chemotherapy/radiation ($p = 0.006$ for PFS, and $p \leq 0.001$ for OS) and American Spinal Injury Association grade C or better at presentation ($p = 0.043$ for PFS, and $p < 0.001$ for OS). There were no significant differences in outcomes based on tumor location, extent of resection, sex, or H3 K27M mutation status. Analysis restricted to HGSG containing necrosis and/or microvascular proliferation (WHO grade IV histological features) revealed increased OS for patients with H3 K27M mutation–positive tumors ($p = 0.017$).

**CONCLUSIONS** Although H3 K27M mutant–positive HGSG was associated with poor outcomes in adult patients, the outcomes of patients with H3 K27M mutant–positive HGSG were somewhat more favorable compared with those of their H3 K27M mutant–negative HGSG counterparts. Further preclinical animal studies and larger clinical studies are needed to further understand the age-dependent effects of H3 K27M mutation.

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**KEYWORDS** H3 K27M; histone H3; spinal cord; glioma; mutation; glioblastoma; oncology; multicenter

**ABBREVIATIONS** ASIA = American Spinal Injury Association; ERC = extensive resection of contrast enhancement; HGSG = high-grade spinal glioma; IDH = isocitrate dehydrogenase; IHC = immunohistochemical; IQR = interquartile range; OS = overall survival; PFS = progression-free survival; STR = subtotal resection; TMZ = temozolomide.

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Diffuse high-grade glioma of the spinal cord is rare, with an age-adjusted incidence as low as 0.22–0.24 per 100,000 person-years.[1,2] The median survival time of patients with these malignant tumors is reportedly 16–24 months.[3,4] Due to the rarity of this tumor, there are few reports of patients with high-grade spinal glioma (HGSG) in the literature, and these are mostly limited to case reports and small case series from single institutions.[5–7] The significance of variables, such as extent of resection and pathological subclassification, remains controversial. H3 K27M mutation refers to a recurrent missense mutation that substitutes methionine for lysine at amino acid position 27 on the tail of one of the histone H3.3 or H3.1 variants that is encoded by the *H3F3A*, *HIST1H3B*, and *HIST1H3C* genes.[8,9] H3 K27M mutation is well described as a poor prognostic indicator for pediatric patients with diffuse midline glioma of the brainstem, thalamus, and spinal cord.[10] The 2016 WHO classification update identified "diffuse midline glioma, H3 K27M–mutant" as a distinct grade IV tumor entity that is biologically divergent from other glioma subtypes owing to its genetic alterations, epigenetic signature, transcriptional profile, cell of origin, and clinical outcomes.[11]

Although conclusive evidence demonstrates that H3 K27M mutation in pediatric patients with diffuse midline glioma is invariably associated with poor prognosis, there is limited evidence to confirm or refute the prognostic association of this mutation in adults with HGSG.[10,12–14] Not only are there limited data regarding the prognostic value of this mutation, but the available data are conflicting. Some studies indicate that H3 K27M mutation is associated with more favorable outcomes,[15] but other reports indicate that the mutation either has no significant effect on prognosis[16] or is in fact a poor prognostic indicator.[17,18] The lack of conclusive evidence on this topic stems from the rarity of the disease and the fact that these histone H3 mutations were discovered less than a decade ago. So far, the only study to suggest more favorable survival in adult patients with H3 K27M mutation–harboring HGSG is from Yi et al.[15] This study included patients from a single institution in South Korea, and thus it may have limited external validity. Based on these conflicting results, we sought to further study the effect of this mutation on prognosis of adult patients with HGSG. The purpose of our study was to describe the prognosis of adult patients with HGSG, with and without H3 K27M mutation, by utilizing a cohort of patients treated at multiple tertiary care academic institutions.

## Methods

### Patient Population

After obtaining IRB approval, we retrospectively reviewed the medical records of patients with histopathologically and pathologically confirmed HGSG who were treated at six tertiary academic centers (Mayo Clinic, Jacksonville, Florida; Mayo Clinic, Rochester, Minnesota; Mayo Clinic, Phoenix, Arizona; Columbia University, New York, New York; Johns Hopkins Hospital, Baltimore, Maryland; and University of California, San Francisco, California) between January 2000 and July 2020. The inclusion criteria were as follows: 1) patient had histopathological confirmation of diffuse glioma of the spinal cord with high-grade histological features (WHO grade III or IV); 2) tumor was tested for H3 K27M mutation status with either sequencing or immunohistochemical (IHC) analysis using a mutant-specific antibody; 3) patient was older than 18 years; 4) preoperative MRI data were available; and 5) patient had available medical records. We excluded all cases of HGSG that were not tested for H3 K27M status.

The medical, radiographic, surgical, and pathological records were reviewed. Medical information analyzed included age at presentation, symptoms at presentation, adjuvant therapy administered, and survival outcomes. The primary outcome variables included progression-free survival (PFS) and overall survival (OS). Secondary variables assessed were preoperative neurological deficit, American Spinal Injury Association (ASIA) status, extent of resection, use of radiation, and chemotherapy treatment received.

### Pathological Assessment

Each case was reviewed by neuropathologists at the included institutions and confirmed as diffuse high-grade astrocytic glioma of the spinal cord. The presence of the H3 K27M mutation was analyzed using IHC staining and pyrosequencing, as previously described (Fig. 1).[14,19] Assessment of H3 K27M status had been performed on a prospective clinical basis for those patients who underwent surgical intervention from 2015 through the present. Retrospective tissue analysis was used to determine H3 K27M mutation status of patients who underwent surgical intervention before 2015.[11] To accurately assess our cohort according to the updated WHO classification, we performed a subgroup analysis of the patients with grade IV tumor according to the 2016 WHO classification update.[11]

### Treatment Modalities

Details regarding extent of surgical resection were extracted by reviewing the operative reports and postoperative images. Patients with intraoperative attestation of complete resection and radiographic confirmation of complete resection of the contrast-enhancing component were regarded as having extensive resection of contrast enhancement (ERC). Because these tumors are known to be infiltrative, it is likely that the true margins of the tumor extended beyond the area of enhancement; thus, it would be difficult to ascertain whether any cases had true gross-total resection. Extent of resection was documented as ERC, subtotal resection (STR), or biopsy. Use of chemotherapy and radiation was extracted and documented. The radiation treatment margins typically included the tumor bed according to findings on postoperative MRI. Patients treated with concurrent temozolomide (TMZ) and radiation were treated according to the typical Stupp regimen protocol for intracranial glioblastoma.[20]

### Statistical Analysis

Survival functions were estimated using the Kaplan-Meier method, and differences were analyzed with

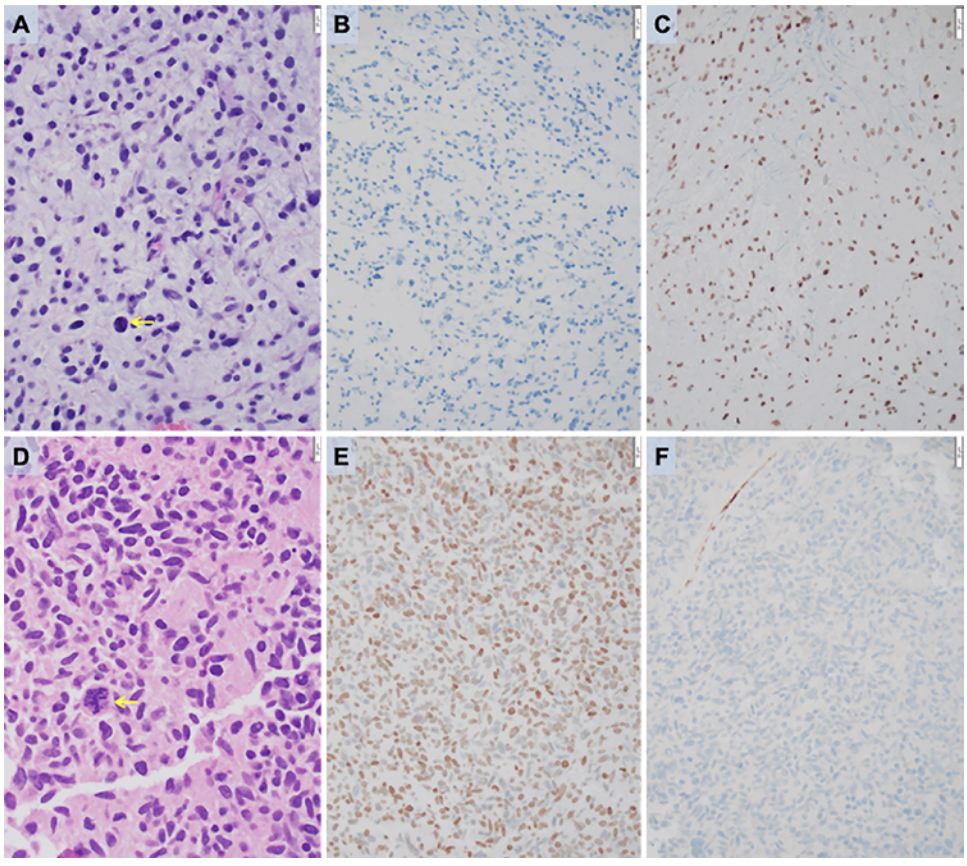


**FIG. 1.** Histological images of diffuse high-grade gliomas of the spinal cord. **Upper:** A 51-year-old woman had a diffuse astrocytic glioma with WHO grade III histological features (i.e., wild-type IDH status, negative results for H3 K27M mutant protein, and histone H3 lysine 27 trimethylation [H3 K27me3] expression). H & E stain shows a mitotically active, infiltrating astrocytic glioma with a mitotic figure (*arrow*, **A**). IHC stain for the H3 K27M mutant protein is shown (**B**). IHC stain for H3 K27me3 shows retained expression (**C**). **Lower:** A 19-year-old woman with an H3 K27M mutant–positive, WHO grade IV diffuse midline glioma. H & E stain shows a mitotically active, infiltrating astrocytic glioma with a mitotic figure (*arrow*, **D**). IHC stain shows expression of the H3 K27M mutant protein (**E**). IHC stain shows loss of H3 K27me3 expression in tumor nuclei, but expression was retained in nonneoplastic endothelial cell nuclei that served as an internal positive control (**F**). Panels A and D, original magnification ×400. Panels B, C, E, and F, original magnification ×200. Figure is available in color online only.

the log-rank test using SPSS software version 1.0.0.1406 (IBM Corp.). Two-tailed $p < 0.05$ was considered significant. Multivariate analysis of the entire cohort was performed using SPSS software. Multivariate analysis of the limited subgroup of patients with grade IV tumor was not possible owing to the small sample size.[21]

## Results

### Patient Population and Clinical Presentation

Thirty patients (18 males and 12 females) with a median (range) age of 50.5 (19–76) years at time of diagnosis were included. Tumors were located in the cervical (n = 7), cervicothoracic (8), thoracic (9), and thoracolumbar (6) spinal cord. All patients underwent preoperative diagnostic MRI for tumor delineation (Fig. 2). Eighteen tumors harbored the H3 K27M mutation, but 12 had mutation-negative results. The patients in the H3 K27M mutation–positive group were significantly older than those in the H3 K27M mutation–negative group (median 55.5 vs 33 years, $p = 0.011$). However, we did not find any statistically significant differences between the groups in regard to patient sex ($p = 0.260$), proportions who received adjuvant chemotherapy and radiotherapy ($p = 0.433$), and proportions who underwent ERC ($p = 0.632$).

The characteristics of all included patients are summarized in Table 1. Of note, this table lists the histological grade that would have been given to these tumors, in addition to H3 K27M mutation status. However, the 2016 WHO update classifies diffuse midline glioma with H3 K27M mutation as WHO grade IV, regardless of histological grade. Motor deficit was the most common presenting symptom in patients with either mutation status, including 12 (66.7%) patients with H3 K27M mutation–positive tumor and 8 (66.7%) patients with wild-type tumor (Table 2).

### Management and Survival of All Included Patients

Ten (33.3%) patients underwent biopsy, 16 (53.3%) underwent STR, and 4 (13.3%) underwent ERC. Twenty-one (70.0%) patients received adjuvant radiotherapy and

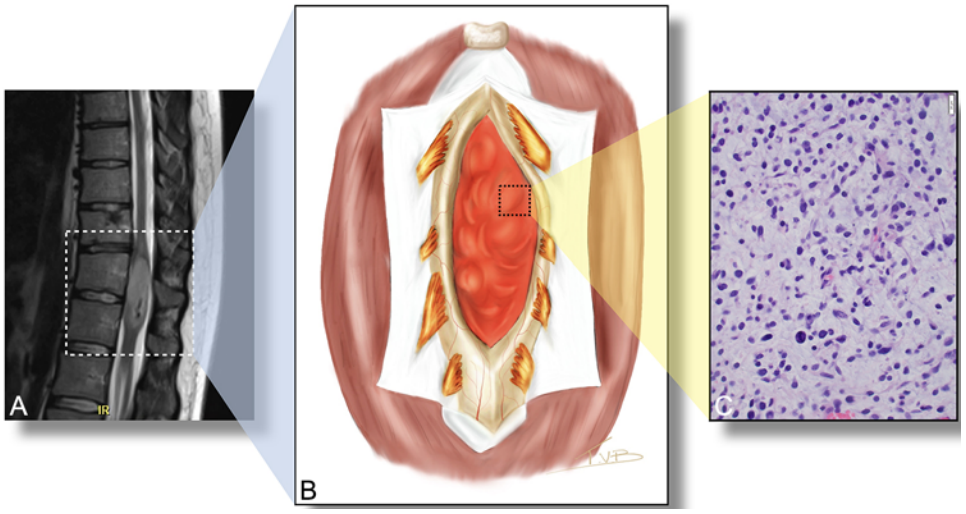


**FIG. 2.** Images and illustrations of diffuse midline gliomas of the spinal cord. **A:** Sagittal T1-weighted postcontrast MR image depicting a thoracic intramedullary tumor. **B:** Artistic rendition of an intramedullary tumor consistent with diffuse HGSG. **C:** H & E stain of a mitotically active, infiltrating astrocytic glioma. Original magnification ×200. Copyright Tito Vivas-Buitrago. Published with permission. Figure is available in color online only.

chemotherapy. Three of 4 patients who underwent ERC had H3 K27M mutation–positive tumor, and 1 had mutation-negative tumor. Of these patients with H3 K27M mutation–positive tumor who underwent ERC, one was a 30-year-old male with a tumor of the conus. Another patient was a 50-year-old female with an H3 K27M mutation–positive tumor with an enhancing border on imaging; the apparent margin was also noted intraoperatively. The third patient was a 76-year-old female with grade IV H3 K27M–negative tumor of the conus that appeared to be well delineated from normal tissue on imaging. The fourth patient was a 31-year-old male with an H3 K27M mutation–positive tumor and an intraoperatively identified border. Postoperative imaging was used to confirm ERC in these patients. TMZ was administered to 17 patients (56.7%), 11 of whom received TMZ as a stand-alone chemotherapeutic agent. Other adjuvant agents included bevacizumab, bortezomib, panobinostat, carboplatin, nivolumab, ipilumab, and imatinib (Table 1).

As of July 2020, the median (interquartile range [IQR]) PFS of all patients in this series was 3 (10) months, and the median (IQR) OS was 9 (23) months. Four patients had OS longer than 3 years. The longest surviving patient was a 56-year-old man with a WHO grade III, wild-type H3 K27M tumor at T11–L2. This patient underwent STR, followed by adjuvant treatment with radiation and TMZ plus bevacizumab. The patient had a PFS of 84 months and an OS of 139 months. Radiographic review revealed that most tumors with the H3 K27M mutation had minimal patchy enhancement, whereas wild-type tumors seemed more likely to have prominent enhancement (Fig. 3). However, this was not a primary outcome of this study and requires analysis with studies focused on radiographic analysis of these tumor types.

Patients who were treated with concurrent chemotherapy and radiation had significantly increased PFS ($p = 0.006$) and OS ($p < 0.001$) when compared with those who did not receive treatment. Neurological status at presentation was also a significant predictor of outcome, and patients who presented with ASIA grade A or B tumor had significantly shorter PFS ($p = 0.043$) and OS ($p < 0.001$). Univariate analysis revealed no significant differences in PFS and OS between patients with H3 K27M mutation–positive tumors and those with wild-type tumors ($p = 0.450$ for PFS, and $p = 0.329$ for OS), between male and female patients ($p = 0.727$ for PFS, and $p = 0.767$ for OS), between patients with cervically located tumors and those with tumors in other locations ($p = 0.103$ for PFS, and $p = 0.223$ for OS), and between patients who underwent ERC and those who underwent STR or biopsy ($p = 0.601$ for PFS, and $p = 0.254$ for OS). Multivariate analysis revealed nonsignificant associations between PFS and age ($p = 0.679$) and mutation status ($p = 0.412$), as well as between OS and age ($p = 0.975$) and mutation status ($p = 0.405$).

### Management and Survival of Patients With WHO Grade IV Tumor

We performed a subgroup analysis of patients with WHO grade IV tumors, including all patients with H3 K27M mutation–positive tumors regardless of histological features according to the 2016 WHO classification guidelines; analysis of patients with H3 K27M mutation–negative tumors was limited to those with necrosis and/or microvascular proliferation. Six patients with WHO grade IV tumors in the wild-type group met the criteria for this analysis. The median age of the wild-type group was 59 years and that of the H3 K27M mutation–positive group was 33 years ($p = 0.040$). We did not find any statistically significant difference between groups with regard to sex ($p = 0.178$), proportion who received adjuvant chemotherapy and radiotherapy ($p = 0.120$), and proportion who underwent ERC ($p = 0.999$).

**TABLE 1. Characteristics and outcomes of patients with spinal diffuse midline glioma**

| Pt No. | Age (yrs), Sex | Location | Histological Grade | H3 K27M Status | IDH1/2 Status | Surgical Intervention | Adjuvant Radiotherapy | Adjuvant Chemo | PFS (mos) | OS (mos) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 19, F | T1–6 | IV | Positive | Wild type | Biopsy | Yes* | TMZ | 1.0 | 3.0† |
| 2 | 58, F | C1–T2 | IV | Positive | Unknown | Biopsy | No | No | 0.1 | 0.1† |
| 3 | 69, F | C3–T5 | IV | Positive | Unknown | Biopsy | No | No | 2.0 | 2.0† |
| 4 | 33, M | C2 | IV | Positive | Wild type | STR | Yes | TMZ & panobinostat | 11.0 | 18.0† |
| 5 | 30, F | T12–L1 | IV | Positive | Wild type | STR | Yes | TMZ & bevacizumab | 10.0 | 27.0† |
| 6‡ | 30, M | T9–L1 | IV | Positive | Unknown | ERC | Yes§ | Panobinostat & bortezomib | 4.0 | 52.0 |
| 7‡ | 50, F | C3–7 | IV | Positive | Unknown | ERC | Yes¶ | Panobinostat | 25.0** | 25.0 |
| 8 | 25, F | T10–11 | IV | Positive | Wild type | STR | Yes¶ | TMZ | 6.0 | 6.0 |
| 9 | 33, M | T11–12 | III†† | Positive | Unknown | STR | Yes¶ | TMZ | 13.0 | 18.0† |
| 10 | 20, M | T10–L2 | III†† | Positive | Unknown | STR | No | NA | 1.0 | 1.0† |
| 11 | 71, M | C7–T1 | III†† | Positive | Unknown | Biopsy | Yes¶ | TMZ | 3.0 | 20.0† |
| 12 | 52, M | T10–L1 | IV | Positive | Wild type | STR | Yes* | Carboplatin, nivolumab, & ipilumab | 0.1 | 18.0† |
| 13‡ | 19, M | T9–10 | IV | Positive | Unknown | Biopsy | Yes§§ | Lomustine & bevacizumab | 16.0 | 20.0† |
| 14‡ | 71, M | T11–12 | IV | Positive | Unknown | STR | Yes | TMZ | 7.0 | 21.0 |
| 15 | 25, M | T6–10 | IV | Positive | Wild type | STR | Yes¶¶ | TMZ | 1.0 | 6.0† |
| 16 | 41, M | C7 | IV | Positive | Wild type | Biopsy | Yes[a] | TMZ & bevacizumab | 1.0 | 7.0† |
| 17 | 71, M | C1–3 | IV | Positive | Unknown | Biopsy | No | NA | NA | 2.0† |
| 18‡ | 31, M | C3–T3 | IV | Positive | Unknown | ERC | Yes[b] | TMZ | 2.0 | NA |
| 19 | 43, M | C4–T1 | IV | Negative | Unknown | Biopsy | No | No | 1.0 | 1.0† |
| 20‡ | 76, F | T12–L1 | IV | Negative | Wild type | ERC | Yes¶ | TMZ | 1.0 | 3.0† |
| 21 | 75, M | T1–2 | IV | Negative | Wild type | STR | No | NA | 1.0 | 1.0† |
| 22 | 72, M | C6–7 | III | Negative | Unknown | Biopsy | Yes¶ | TMZ | 24.0 | 39.0 |
| 23 | 44, M | C3–8 | III | Negative | Wild type | STR | Yes¶ | TMZ | 13.0 | 27.0† |
| 24 | 51, F | C4–T1 | III | Negative | Wild type | STR | Yes | TMZ | 6.0 | 9.0 |
| 25 | 56, M | T11–L2 | III | Negative | Unknown | STR | Yes¶ | TMZ & bevacizumab | 84.0 | 139.0† |
| 26 | 50, M | C7–T2 | IV | Negative | Unknown | STR | Yes | TMZ & imatinib | NA | NA |
| 27‡ | 64, M | C2–3 | III | Negative | Unknown | STR | Yes | TMZ & bevacizumab | 3.0 | 72 |
| 28 | 55, M | C6–T2 | IV | Negative | Wild-type | STR | No | NA | 1.0** | 1.0† |
| 29 | 53, F | T3–5 | III | Negative | Unknown | STR | NA | NA | NA | NA |
| 30‡ | 63, M | T1–4 | IV | Negative | Unknown | Biopsy | No | NA | 2.0 | 2.0 |

Chemo = chemotherapy; NA = not applicable; pt = patient.
* The patient received 45 Gy in 25 fractions.
† The patient died.
‡ The patient was included in a previously published study (Sloan et al., 2019[38]).
§ The patient receive 36 Gy in 12 fractions.
¶ The patient received 54 Gy in 30 fractions.
** The patient was progression free at the time of analysis.
†† WHO grade IV according to the updated 2016 WHO guidelines.
§§ The patient received 41 Gy.
¶¶ The patient received 48 Gy in 26 fractions.
[a] The patient received 48 Gy in 27 fractions.
[b] The patient received 22 Gy in 5 fractions.

Interestingly, patients with H3 K27M mutation–positive tumors had increased OS ($p = 0.017$). Figure 4 depicts the Kaplan-Meier curves of PFS and OS for the patients included in this study. Patients who received chemotherapy and radiation had significantly increased OS ($p \leq 0.001$) compared with those patients who did not, but rates of PFS were similar ($p = 0.117$). Patients who presented with poor neurological status (ASIA grade A/B) had significantly worse OS ($p = 0.003$) compared with patients who presented with ASIA grade C or better. Table 3 summarizes the results of univariate analysis of all included patients, as well as the analysis of WHO grade IV tumor.

## Discussion

This is the first multicenter assessment of the prognostic value of H3 K27M mutation for adult patients with HGSG. Our understanding of the significance of H3 K27M muta-

**TABLE 2. Clinical data of patients with spinal diffuse midline glioma**

| Characteristic | All Pts (n = 30) | H3 K27M Positive (n = 18) | H3 K27M Negative (n = 12) | p Value |
|---|---|---|---|---|
| Pain | 8 (26.7) | 4 (22.2) | 4 (33.3) | 0.678 |
| Motor deficit | 20 (66.7) | 12 (66.7) | 8 (66.7) | 0.999 |
| Sensory deficit | 8 (26.7) | 7 (38.9) | 1 (8.3) | 0.099 |
| Bowel & bladder impairment | 6 (20.0) | 4 (22.2) | 2 (16.7) | 0.999 |
| ASIA grade | | | | |
| A/B | 5 (16.6) | 2 (11.1) | 3 (25.0) | 0.364 |
| C/D | 20 (66.7) | 13 (72.2) | 7 (58.3) | 0.461 |

Values are shown as number (percent) unless indicated otherwise.

tion in adults is limited, with data supporting lack of benefit, benefit, and no significant prognosticative value. This study shows that although all patients with HGSG had poor prognosis, patients with H3 K27M mutation had increased OS ($p = 0.017$) compared with those patients with negative mutation status. Of note, patients with H3 K27M–negative tumor were older than patients with mutation-positive tumor (median 59 vs 33 years, $p = 0.040$), which may have contributed to this difference. Our results showed a high frequency of H3 K27M mutation in patients with HGSG, with 60% of our cohort harboring the mutation. We also confirmed previously reported data showing that neurological status at prognosis and that use of chemotherapy and radiation had significant effects on prognosis in these patients.

Previously published data by Yi et al. showed that presence of H3 K27M mutation was actually a positive predictive factor for adult patients with HGSG.[15] Prior to this, the only available studies in the literature that analyzed the role of this mutation indicated that H3 K27M mutation–positive tumor is uniformly fatal with poor prognosis, although these studies were conducted with pediatric

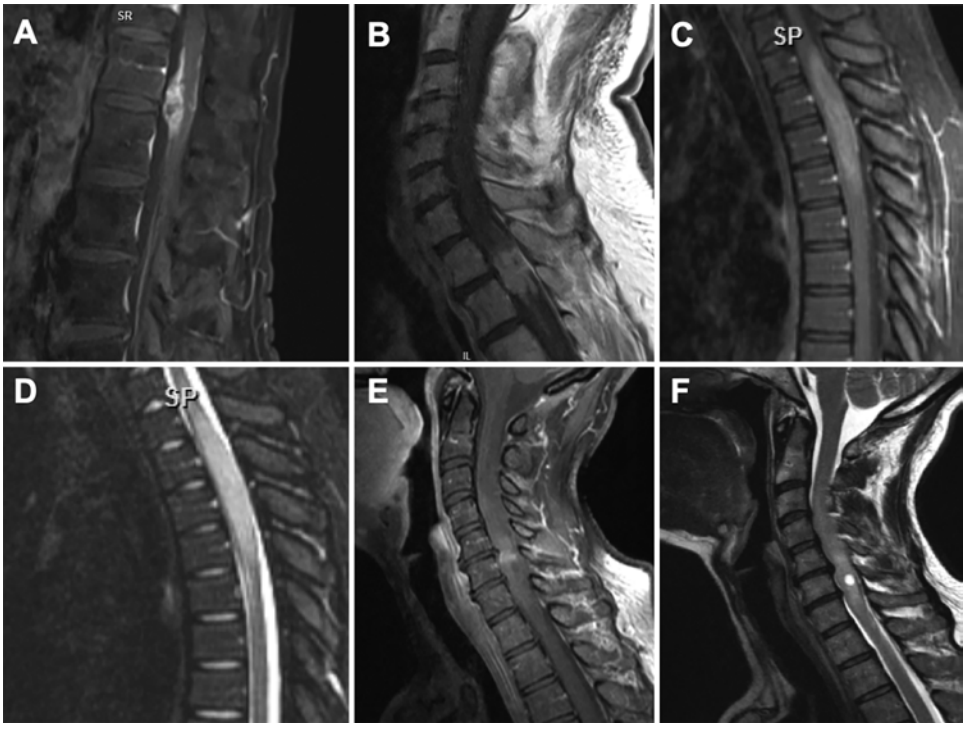


**FIG. 3.** MR features of diffuse HGSGs. **A:** Sagittal T1-weighted postcontrast MR image demonstrating robust enhancement of an intramedullary tumor centered at T12–L1 in a 76-year-old woman. She underwent resection, and WHO grade IV, H3 K27M mutation–negative glioblastoma was confirmed. **B:** Sagittal T1-weighted postcontrast MR image depicting an intramedullary tumor centered at T1–2 in a 75-year-old man. He underwent STR, and a WHO grade IV, H3 K27M mutation–negative glioblastoma was confirmed. **C and D:** Sagittal T1-weighted postcontrast and T2-weighted MR images of a 19-year-old woman with a WHO grade IV, H3 K27M mutation–positive diffuse midline glioma extending at T1–T6. **E and F:** Sagittal T1-weighted postcontrast and T2-weighted images demonstrating minimal enhancement of an intramedullary tumor centered at C3–6 in a 50-year-old man. Pathological analysis demonstrated a WHO grade IV, H3 K27M mutation–positive diffuse midline glioma.

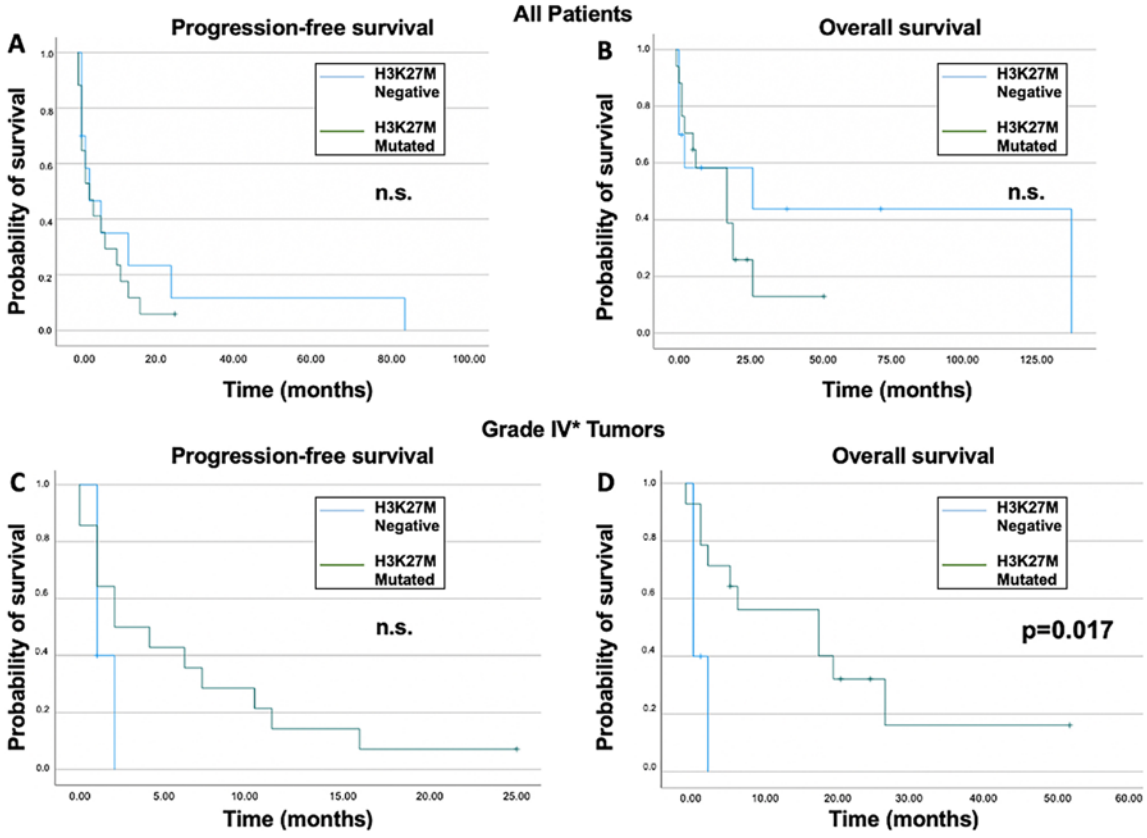


**FIG. 4.** Clinical outcomes of adult patients with diffuse HGSG, stratified according to H3 K27M mutation status. **A and B:** Kaplan-Meier curves of all patients included in our cohort, comparing PFS and OS between patients with H3 K27M mutation–positive tumors and those with H3 K27M–negative tumors. **C and D:** Kaplan-Meier curves of the subset of patients with WHO grade IV gliomas, comparing PFS and OS (p = 0.017) between patients with H3 K27M mutation–positive tumor and those with H3 K27M–negative tumor. *Grade IV was determined according to the 2016 WHO classification. n.s. = not significant. Figure is available in color online only.

populations.[22,23] The study by Yi et al. was the first and largest study to assess the role of this mutation in adult patients with HGSG, as well as the first study to analyze such patients according to the updated WHO classifications. Our finding that H3 K27M mutation was associated with poor prognosis, as well as that patients with this mutation had somewhat more favorable outcomes than patients with H3 K27M–negative glioblastoma of the spinal cord, supports recent findings in the literature that indicate different effects of this mutation on outcomes between adult and pediatric patients with HGSG.[15,16] Our results add to the growing body of studies indicating heterogeneity in the prognostic significance of H3 K27M mutation for patients with various ages and tumor locations.

The role of extent of resection remains controversial for patients with HGSG. Our results also indicate that extent of resection does not play a role in the prognosis of patients with spinal cord glioblastoma. Nunna et al.[3] analyzed National Cancer Database records of 396 patients with HGSG. They reported a mean OS of 24.5 months and found no association between extent of resection and overall prognosis; however, multiple studies have found that radical resection results in increased survival for patients with HGSG.[4,24,25] At this time, no consensus can be made regarding the role of extent of resection for patients with HGSG, given the conflicting results to date.[6,26] We were unable to directly analyze the effect of tumor location and H3 K27M mutation status on extent of resection, and this observation would need to be validated with a larger study.

HGSG harbors a diverse population of mutations and genetic aberrations, which is also typical of its supratentorial counterpart.[7,27,28] This provides the opportunity to target these pathways using chemotherapeutic agents. Panobinostat is a nonselective histone deacetylase inhibitor, and small series on this drug have shown early promise for treating patients with H3 K27M–positive glioma.[29,30] Our series included only 3 patients who were treated with panobinostat, so we were unable to draw conclusions regarding the overall benefit of the drug. However, the mean PFS was 11.6 months for these 3 patients treated with panobinostat and 10.6 months for those who were not. There is an ongoing phase I clinical trial on panobinostat as a treatment of children with diffuse intrinsic pontine glioma (ClinicalTrials.gov: NCT02717455).

Over the past several years, animal models have been created to further study the malignant effects of H3 K27M mutation, including a genetically engineered mouse mod-

**TABLE 3. Univariate analysis of prognostic factors for patients with spinal diffuse midline glioma**

| Characteristic | All Pts | | | | Pts w/ WHO Grade IV Glioma | | | |
|---|---|---|---|---|---|---|---|---|
| | PFS (mos) | p Value | OS (mos) | p Value | PFS (mos) | p Value | OS (mos) | p Value |
| Sex | | 0.727 | | 0.767 | | 0.698 | | 0.989 |
| Male | 3 | | 18 | | 2 | | 7 | |
| Female | 4 | | 4.5 | | 2 | | 3 | |
| H3 K27M status | | 0.450 | | 0.329 | | 0.239 | | **0.017** |
| Negative | 2.5 | | 6 | | 1 | | 1.5 | |
| Positive | 3 | | 18 | | 3 | | 12.5 | |
| Location | | 0.103 | | 0.223 | | 0.100 | | 0.813 |
| Cervical | 12 | | 25 | | 11 | | 12.5 | |
| Noncervical | 2 | | 6 | | 2 | | 4.5 | |
| Extent of resection | | 0.601 | | 0.254 | | 0.372 | | 0.103 |
| ERC | 3 | | 25 | | 3 | | 25 | |
| STR or biopsy | 3 | | 8 | | 1.5 | | 6 | |
| Radiation/chemo | | **0.006** | | **<0.001** | | 0.117 | | **<0.001** |
| Yes | 6 | | 20 | | 4.5 | | 18 | |
| No | 1 | | 1 | | 1 | | 1 | |
| ASIA grade | | **0.043** | | **<0.001** | | 0.218 | | **0.003** |
| A/B | 1 | | 1 | | 1 | | 1 | |
| C/D | 5 | | 18 | | 2 | | 6.5 | |

Median values are shown unless indicated otherwise. Boldface type indicates statistical significance ($p < 0.05$).

el, replication-competent avian sarcoma-leukosis virus–tumor virus A (RCAS-TVA) model, and patient-derived xenografts.[31–33] Deeper insights into the molecular mechanisms of pathogenesis are being revealed with these studies; however, this work needs to be replicated and verified in a larger cohort of patients with these tumors. These experiments also need to be replicated with tissues from adult patients in order to further understand the mechanisms that drive these differences in effects between adults and children. As we broaden our understanding of these tumors, hopefully we will be able to develop targeted therapy against this mutation.

### Strengths and Limitations

Our study was limited by its retrospective nature and number of included patients. The limited number of patients is an inherent limitation of this disease process due to its rarity. This led to a limited number of patients available for comparison of WHO grade IV tumors. Another limitation of our study is the lack of complete assessments of isocitrate dehydrogenase (IDH) status, which is a known predictor of outcome in patients with intracranial glioblastoma.[34] Multiple studies have shown that IDH mutations are exceedingly rare in spinal gliomas; therefore, IDH status was very unlikely to be a confounding factor in this study.[35–37] We were unable to analyze our cohort for other genetic markers, and it is possible that our results could have been confounded by other mutations that were not tested in this patient cohort.

In this study, IHC analysis was the principal method used to determine histone H3 mutation status. Although IHC analysis using an H3 K27M mutation–specific antibody is a clinically accepted, standard-of-care method for assessment of H3 K27M mutation status, this methodology only identifies those gliomas harboring H3 K27M mutation. A recent genomic evaluation of diffuse spinal cord glioma identified *H3F3B* p.K27I and *H3F3A* p.G34W mutations in those tumors lacking the canonical H3 K27M mutation, but neither of these mutations is detected with the H3 K27M mutation–specific antibody.[38] Sequencing all histone H3 genes is not standard of care, but this would be necessary to identify these other less frequent mutations. In our study, we performed multivariate analysis to evaluate the entire cohort, but we were unable to perform a multivariate analysis of grade IV tumors owing to the limited sample size. This was the first multicenter analysis of patients with HGSG, and we included patients from five different geographical regions of the United States (Northeast, Southeast, Midwest, Southwest, and West). Thus, a strength of this study is its excellent external validity due to the geographic representation.

## Conclusions

This was the first multicenter study to assess the role of H3 K27M mutation in adult patients with HGSG. This study revealed that all spinal cord glioblastomas are associated with poor prognosis, but patients with H3 K27M mutant–positive tumor appeared to have a better prognosis than those with H3 K27M–negative tumor. Also, our study showed that H3 K27M mutation appears to be frequently encountered in patients with HGSG, with 60% of our cohort harboring this mutation. Future studies with larger patient cohorts are needed to evaluate the prognos-

tic effect of H3 K27M mutation and correct for potential confounding variables.

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## Disclosures

Dr. Kalani is a consultant for Medtronic, NuVasive, and CarboFix. Dr. Sciubba is a consult for DePuy-Synthes, Medtronic, Stryker, and Baxter. Dr. Clarke is a consultant for Agios; and receives non–study-related clinical or research effort overseen by the author from Agios and Merck.

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Conception and design: Akinduro, Higgins. Acquisition of data: Akinduro, Garcia, Higgins, Pennington, Delgardo. Analysis and interpretation of data: Akinduro, Garcia. Drafting the article: Akinduro, Garcia, Higgins, Vivas-Buitrago, Jentoft. Critically revising the article: Akinduro, Garcia, Solomon, Daniels, Sherman, Bydon, Kalani, Abode-Iyamah, Quiñones-Hinojosa. Reviewed submitted version of manuscript: Akinduro, Garcia, Higgins, Jentoft, Solomon, Daniels, Sherman, Bydon, Kalani, Zanazzi, Tsankova, Bendok, McCormick, Sciubba, Lo, Clarke, Abode-Iyamah, Quiñones-Hinojosa. Approved the final version of the manuscript on behalf of all authors: Akinduro. Statistical analysis: Akinduro. Study supervision: Abode-Iyamah, Quiñones-Hinojosa.

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