Insights From H3-Wildtype Diffuse Midline Glioma With EZHIP Overexpression

To the Editor:

Diffuse midline glioma (DMG) H3K27-altered presents a formidable challenge in neuro-oncology due to its aggressive nature and predilection for midline structures. The 2021 WHO Classification of Tumors has delineated subtypes of DMG, characterized by H3K27 mutation (DMG-H3.3 K27 and DMG-H3.1/3.2 K27), H3-wildtype with EZHIP overexpression (DMG-EZHIP), and *EGFR* alterations (DMG-EGFR), with the shared feature of H3K27me3 loss.[1] We read Zheng et al's article[2] published in the *American Journal of Surgical Pathology* in 2022, which reported a cohort of 94 adult and 70 pediatric patients diagnosed with DMG with H3K27M mutation. The study revealed a median survival time of 5.0 months for pediatric patients and 16.0 months for adults ($P=0.0003$), along with other significant differences in tumor location and molecular characteristics. To provide further insights and comparisons within the spectrum of DMG, we present a consecutive cohort of 16 H3-wildtype DMG patients with EZHIP overexpression, including 9 pediatric patients (aged under 18 y) and 7 adults. The DNA-based targeted next-generation sequencing (NGS) was retrospectively conducted in all cases, and ethical approval was obtained from the Ethics Committee of Beijing Tiantan Hospital. Pathologic diagnosis was performed by senior pathologists, confirming H3K27me3 (−), H3K27M (−), and EZHIP (+) through immunohistochemical analysis (Fig. 1D).

This cohort demonstrated a median overall survival (OS) of 11.0 months (95% CI: 0.00-23.81), similar to 10.5 months in DMG-H3K27M[2] and 11.0 months in diffuse intrinsic pontine glioma (DIPG).[3] Kaplan-Meier analysis revealed that adjuvant therapy generated an improved OS (Log-rank $P=0.006$) (Fig. 1A). Patients who failed to receive adjuvant treatment typically faced rapid tumor progression, with a median OS of 1.0 month (95% CI: 0.00-3.15), thereby limiting opportunities for further therapeutic intervention. This finding aligns with conclusions from a large cohort study of DIPG, which also reported a median OS of 1.0 months for untreated patients.[3] The correlation between longer OS and early therapeutic intervention underscores the aggressive nature of DMG across its subtypes. No discernible gender preference was observed (male-to-female ratio = 1:1), consistent with the gender distribution seen in DMG-H3K27M cases.[2,4]

A pioneering study indicates that the most commonly observed genetic variations in DMG-EZHIP are *ACVR1*(14/19), followed by *PIK3CA*, *TP53*, and *PPM1D*.[5] Based on NGS (Fig. 1B), *TP53* alterations (8/16, 50.00%) were the most prevalent genetic alterations in this cohort, which aligns with observations in DMG with H3K27M mutation.[4] The *ACVR1* alterations (4/16, 25.00%) were exclusively identified in the pediatric group, with p.G328E found in 2 cases, p.R258G in 1 case, and concurrent p.R375H and p.G328V in another. This finding correlates with the reported association between *ACVR1* alterations and younger age in DIPG.[6] Furthermore, cases 1 and 13 exhibited mutations in both *EGFR* and *ACVR1*, expanding upon the reported hypothesis of a mutually exclusive relationship between mutations in these 2 genes in H3-wild type DMG,[7] as observed in a larger sample size.

Previous studies have indicated that when *EGFR* mutations and EZHIP overexpression occur concurrently, the methylation cluster analysis suggests an EGFR-mutant subtype.[8,9] This implies that for H3-wildtype DMG, subtyping cannot be fully determined merely by EZHIP immunohistochemical staining. Instead, *EGFR* mutations may play a more crucial role, and DNA methylation exerts significant influence in the classification process. Within the presented cohort, the *EGFR* alterations were identified in 7 cases (43.75%), including p.G598V (n = 1), p.G601A (n = 1), amplification (n = 1), concurrent p.G598V and amplification (n = 2), and concurrent p.A289V and amplification (n = 2). A comparison with the counterparts demonstrated that the cases featuring *EGFR* alterations exhibited a higher frequency of *TP53* mutation (Supplemental Fig. S1, Supplemental Digital Content 1, http://links.lww.com/PAS/C67). However, no significant differences were observed in OS (Log-rank $P=0.642$) or age ($P=0.615$). The relationship between *EGFR* alterations and bilateral thalamic DMG was also suggested in some reports.[9–11] Four cases with bilateral thalamic tumors were identified in this cohort, and 3 of them (75.00%) had *EGFR* alterations (one with p.G598V, others with concurrent p.A289V and amplification). However, bilateral thalamic tumors did not exhibit a significant difference in OS compared with unilateral counterparts (Log-rank $P=0.989$) (Fig. 1C). Considering the limited sample size, the role of *EGFR* alterations, as well as the impact of genetic alterations with high prevalence on prognosis (Supplemental Table S2, Supplemental Digital Content 2, http://links.lww.com/PAS/C68), are still inconclusive.

The median age at diagnosis was 15.50 years, with 7.00 years (range: 4.00 to 16.00 y) in the pediatric group and 35.00 years (range: 30.00 to 67.00 y) in the adult group (Supplemental Table S1, Supplemental Digital Content 3, http://links.lww.com/PAS/C69). The median OS was 4.00 months (95% CI: 0.00-12.77) for the pediatric group, and 19.00 months (95% CI: 2.31-35.69) for adults, with no significant difference observed ($P=0.093$). The extent of resection differed significantly between age groups ($P=0.024$), with a higher proportion of adults receiving gross total or near total resection (85.71% vs. 11.11%), while more pediatric patients opted for biopsy (55.56% vs. 14.29%). In addition, Cox regression analysis revealed that despite DMG predominantly affecting pediatric populations,