Title: Pre-treatment Monocytosis in Pediatric H3K27M-Mutant Gliomas

Running Head: H3K27M-Mutant Glioma Monocytosis

Authors: Hannah Weiss[1], Eric A. Grin[1], Anfal Alshammari[2,3], Jonathan Serrano[4], Camila Fang[4], Anirban Das[2], Cynthia Hawkins[5], Jessica Clymer[6], Devorah Segal[6], Matthias A. Karajannis[7], David Harter[1], Eveline Teresa Hidalgo[1#], and Matija Snuderl[4,8#]

Affiliations:
[1] Department of Neurosurgery, NYU Langone Health and NYU Grossman School of Medicine, New York, New York, United States
[2] Division of Pediatric Haematology Oncology, Department of Paediatrics, Hospital for Sick Children (SickKids), Toronto, Canada
[3] Department of Pediatric Hematology Oncology, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
[4] Department of Pathology, NYU Langone Health and NYU Grossman School of Medicine; New York, NY 10016, USA
[5] Department of Paediatric Laboratory Medicine, Hospital for Sick Children (SickKids), Toronto, Canada
[6] Department of Pediatrics NYU Langone Health and NYU Grossman School of Medicine; New York, NY 10016, USA
[7]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY 10065, United States
[8] Brain and Spine Tumor Center, Laura and Isaac Perlmutter Cancer Center, New York, NY, USA

[#]Dr. Eveline Teresa Hidalgo and Dr. Matija Snuderl contributed equally to this article and are co-senior authors.

Corresponding Author:
Matija Snuderl, MD
Email: Matija.snuderl@nyulangone.org
Department of Pathology
NYU LangoneHhealth
240 E 38th Street, 22nd Floor
New York, NY 10016
ORCID: 0000-0003-0752-0917

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ABSTRACT

Background: Systemic immune dysregulation is well-characterized in adult high-grade gliomas but remains underexplored in pediatric population. Among these, H3K27M-mutant diffuse midline gliomas are biologically and clinically distinct, with limited therapeutic options and poor prognosis. While prior studies in medulloblastoma suggest tumor-induced lymphopenia at diagnosis, the immune profile of H3K27M gliomas has not been systematically evaluated.

Methods: We performed a multicenter retrospective analysis of pretreatment complete blood counts (CBCs) in pediatric patients (≤18 years) with H3K27M-mutant gliomas (n=37), comparing them to a control cohort with pilocytic astrocytoma (n=61). Absolute neutrophil count (ANC), monocyte count (AMC), lymphocyte count (ALC), neutrophil-to-lymphocyte count ratio (NLCR), and monocyte-to-lymphocyte ratio (MLR) were evaluated. Patients receiving corticosteroids prior to CBC collection were excluded.

Results: No significant differences were observed in ANC, ALC, or NLCR between groups. However, the H3K27M group demonstrated significantly elevated AMC (0.70 vs. 0.50 K/μL; p=0.012) and MLR (0.20 vs. 0.19; p=0.021) compared to controls. Monocytosis, defined by AMC above age-adjusted means, was present in 78.4% of H3K27M patients versus 46.7% of controls (OR=4.14, p=0.003). These findings suggest a distinct pre-treatment immune signature in H3K27M gliomas.

Conclusions: This is the first study to identify pre-treatment monocytosis and elevated MLR in pediatric H3K27M-mutant gliomas, highlighting a novel systemic immune phenotype with potential implications for biomarker discovery and immunotherapeutic targeting. These results underscore the need for further mechanistic studies and prospective validation in larger, mutation-stratified cohorts.

Keywords

Pediatric neuro-oncology, monocytosis, monocyte-to-lymphocyte ratio, systemic immune signature, H3K27M-mutant diffuse midline glioma

Key Points

- Pediatric H3K27M-mutant gliomas exhibit a distinct pre-treatment systemic immune signature characterized by monocytosis and an elevated monocyte-to-lymphocyte ratio.
- Unlike other pediatric brain tumors, this cohort shows a specific myeloid-driven profile.

Importance of the Study

This study provides the first systematic characterization of systemic immune profiles in pediatric H3K27M-mutant diffuse midline gliomas. While prior literature on adult glioblastomas and pediatric medulloblastomas emphasizes tumor-induced lymphopenia, our findings reveal a distinct pre-treatment signature of monocytosis and elevated monocyte-to-lymphocyte ratios in H3K27M-mutant disease. This indicates that systemic immune dysregulation is tumor-type

specific and suggests that H3K27M-mutant diffuse midline gliomas actively modulate the myeloid lineage early in the disease course. These results establish inexpensive, CBC-derived metrics as potential non-invasive biomarkers for clinical risk stratification. Furthermore, the identification of a systemic myeloid phenotype provides a rationale for future mechanistic studies into the myeloid-rich tumor microenvironment and the development of myeloid-targeted immunotherapies for this pediatric population.

MAIN TEXT

Introduction

Primary brain tumors represent the most common group of solid tumors and the leading cause of cancer-related death in children. Tumor-induced systemic immune suppression- characterized by lymphopenia, anergy, CD4+ T-cell depletion, and a cytokine milieu favoring regulatory T cells (Tregs)- has been well-documented in adult high-grade gliomas, even prior to the initiation of cytotoxic therapy.[1-3] In these patients, elevated neutrophil-to-lymphocyte count ratios (NLCRs) have been consistently associated with poorer prognosis.[4, 5]

Pediatric gliomas, however, are biologically distinct from their adult counterparts, often harboring unique genetic and epigenetic alterations. Our recent studies have also demonstrated that pediatric gliomas, regardless of molecular or histological grade share metabolomic features, which are distinct from adult gliomas.[6] Among these, the H3K27M mutation- originally described in diffuse intrinsic pontine gliomas (DIPGs)- but eventually described throughout the CNS[7, 8] is associated with midline CNS localization, rapid progression and poor outcomes.[9] H3K27M mutant gliomas, reclassified by the World Health Organization in 2021 as "diffuse midline glioma, H3K27-altered," are associated with a highly aggressive clinical course and poor prognosis.[10, 11]

We previously observed that pediatric patients with medulloblastoma exhibit lymphopenia at diagnosis, suggesting that tumor-induced immune suppression may occur prior to therapy in pediatric brain tumors as well.[12] To our knowledge, there are no published studies investigating tumor-induced systemic immune suppression at diagnosis in pediatric patients with high-grade gliomas. In this study, we systematically characterize pre-treatment peripheral blood counts in

children with H3K27M-mutant diffuse midline gliomas and compare them with age-matched patients with pilocytic astrocytoma, aiming to identify distinctive systemic immune signatures- particularly aberrations in neutrophil, lymphocyte, and monocyte metrics- that could serve as biomarkers of tumor-induced immune dysregulation and guide future immunotherapeutic strategies.

Pilocytic astrocytoma (PA) served as the control group rather than healthy pediatric patients, as both cohorts represent children with central nervous system tumors undergoing similar diagnostic and pre-treatment evaluations. Although CBC parameters in children with PA are typically within normal limits—since PA is a localized, non-infiltrative neoplasm that rarely affects systemic hematopoiesis—using a tumor-bearing comparison group helps account for potential confounders such as physiologic stress, peritumoral inflammation, and preoperative care factors not present in healthy controls.

Materials and Methods

Patient Selection

We retrospectively identified pediatric patients with H3K27M-mutant high-grade gliomas (H3K27M) from the NYU pediatric neuro-oncology and molecular pathology database and a database from The Hospital for Sick Children (SickKids) in Toronto, Canada. The combined cohort included patients ≤18 years of age diagnosed with an H3K27M-mutant glioma between 2000 and 2024 at either institution. Patients were excluded if a complete blood count (CBC) with

differential prior to any treatment was unavailable or if corticosteroids had been administered before the initial CBC, as corticosteroids can rapidly alter neutrophil and lymphocyte counts.[13]

A control group was composed of patients diagnosed with pilocytic astrocytoma (PA) over the same timeframe, using identical inclusion and exclusion criteria.

The following CBC parameters were retrieved directly from clinical records in both the study and control groups, without additional blood processing or PBMC isolation: ANC, AMC, and ALC. The NLCR was calculated as neutrophil count divided by lymphocyte count, and MLR was calculated as monocyte count divided by lymphocyte count. Relevant demographic and clinical information, including age, sex, and tumor location was also recorded. The study was approved by the Institutional Review Board (NYU IRB#: S14-00948)**.**

Mutation Confirmation

H3K27M status was determined as part of standard clinical care. For NYU cases, H3K27M detection was performed by clinically validated assays including FDA cleared NYU Langone Genome PACT, DNA Methylation profling using Heidelberg CNS Classifier v12 , as previously described,[14, 15] or clinical immunohistochemistry for the H3K27M protein.[15] A similar process was conducted for samples obtained from The Hospital for Sick Children (SickKids), where H3K27M mutation status was confirmed via institutional clinical testing using immunohistochemistry and/or DNA methylation profiling based on available tumor tissue.

Statistical Analysis

Statistical analyses were performed using Python (version 3.12.3) with the scipy.stats and statsmodels libraries. Continuous variables, including age, absolute neutrophil count (ANC), absolute monocyte count (AMC), absolute lymphocyte count (ALC), neutrophil-to-lymphocyte count ratio (NLCR), and monocyte-to-lymphocyte ratio (MLR), were assessed for normality using the Shapiro–Wilk test. Given that all continuous parameters exhibited non-normal distributions, comparisons between the H3K27M and PA cohorts were conducted using the non-parametric Mann–Whitney U test. Results for these variables are reported as medians with interquartile ranges (IQR). The incidence of monocytosis, treated as a binary categorical variable, was defined as an AMC exceeding the age-adjusted reference mean from the CALIPER normative dataset. This threshold was selected in preference to the upper reference limit because our aim was to detect a population-level shift in the myeloid compartment rather than to identify clinically overt monocytosis in individual patients. As a pre-specified sensitivity analysis, monocytosis was additionally classified using published CALIPER upper reference limits (AMC > 1.1 × 10^9/L for ages 0–<5 years; AMC > 0.8 × 10^9/L for ages 5–<21 years). Fisher's exact test was used to determine the odds ratio and associated 95% confidence intervals for both definitions."

Results

Our cohort included 37 children with H3K27M mutant glioma and 61 with PA. The female-to-male ratio was 1.06 (19:18) in the H3K27M cohort and 0.85 (28:33) in the PA cohort. There was no significant difference in age between the H3K27M and Control groups, median age at diagnosis was 8 years (IQR 4–11; n = 37) in the H3K27M cohort and 6 years (IQR 3–10; n = 61) in the PA cohort (p=0.18) (**Table 1**). Of the 37 patients with H3K27M-mutant glioma, anatomical location data were available for 27. The majority (21/27, 78%) involved the midline brainstem (including

pons and midbrain). The remaining cases included left thalamus (n=2), anterior left temporal lobe (n=1), right frontal lobe (n=1), cerebellar hemisphere with pons and midbrain involvement (n=1), and a tectal/midbrain mass (n=1).

All peripheral-blood variables were non-normally distributed and analyzed using the Mann–Whitney U test (Supplemental Tables 1–2). In healthy pediatric populations, the absolute neutrophil count (ANC) generally ranges from 1.5–6.5 × 10^3/μL, the absolute lymphocyte count (ALC) from 2.0–8.0 × 10^3/μL, and the absolute monocyte count (AMC) from 0.3–1.1 × 10^3/μL, with age-dependent shifts in balance between neutrophils and lymphocytes. The neutrophil-to-lymphocyte count ratio (NLCR) typically falls between 0.7 and 3.5, and the monocyte-to-lymphocyte ratio (MLR) between 0.2 and 0.3 in healthy children.

Within this context, both tumor populations in our study showed hematologic profiles broadly comparable to age-adjusted reference values, with median ANC, ALC, and NLCR similar between H3K27M and PA groups (all $p > 0.5$). However, monocyte indices were distinctly elevated in the H3K27M cohort compared to the PA group. The median AMC was 0.70 K/μL in H3K27M versus 0.50 K/μL in PA ($p = 0.012$), and the median MLR was 0.20 in H3K27M compared to 0.19 in PA ($p = 0.021$) (Figure 1). When evaluated against age-adjusted reference means, 78.4% of H3K27M patients met criteria for monocytosis, compared to 46.7% of PA patients (odds ratio 4.14, $p = 0.003$; Figure 2). In a sensitivity analysis using CALIPER upper reference limits as an alternative threshold (AMC > 1.1 × 10^9/L for ages 0–<5 years; AMC > 0.8 × 10^9/L for ages 5–<21 years), monocytosis was present in 16.2% of H3K27M patients (6/37) versus 8.2% of PA patients (5/61; OR=2.17, p=0.32). The primary threshold-independent evidence for a myeloid shift in the H3K27M cohort is provided by the continuous AMC and

MLR comparisons, which remained statistically significant regardless of binary classification threshold (p=0.012 and p=0.022, respectively).

Thus, while both tumor-bearing cohorts demonstrated blood counts largely within expected pediatric ranges, H3K27M-mutant gliomas were characterized by a consistent and statistically significant elevation in monocyte-related parameters, suggesting a distinct systemic monocytic inflammatory signature not observed in low-grade controls.

In exploratory subgroup analyses within the H3K27M cohort, no significant associations were identified between AMC or monocytosis status and tumor location, age at diagnosis, or sex. With respect to tumor location, subgroup analysis was performed using the 26 patients for whom both location and matched clinical data were available; of these, 24 had brainstem tumors (median AMC 0.61 K/μL, IQR 0.48–0.73; monocytosis in 70.8%) and 2 had non-brainstem tumors (median AMC 0.55 K/μL; monocytosis in 50.0%), with no significant difference between groups (Mann-Whitney U p=0.63; Fisher's exact p=0.53). The non-brainstem group comprised only 2 patients, precluding meaningful statistical interpretation by location. No significant correlation was identified between age at diagnosis and AMC across all 37 patients (Spearman rho=−0.167, p=0.32). Regarding sex data, AMC was 0.63 K/μL in males versus 0.60 K/μL in females, with monocytosis present in 76.9% and 61.5% respectively, a difference that did not reach statistical significance (Mann-Whitney p=0.74; Fisher's exact p=0.67). These analyses are limited by sample size and should be considered hypothesis-generating.

In an exploratory survival analysis, overall survival data were available for 24 of the 26 matched H3K27M patients with complete clinical records (20 deceased, 4 alive at last follow-up). Survival time was calculated from the date of qualifying CBC to date of death, with alive

patients right-censored at the date of last known follow-up. Monocytosis-positive patients (n=16) had a median overall survival of 7.8 months (range 0.8–16.4 months), compared with 9.8 months (range 7.4–39.6 months) in monocytosis-negative patients (n=8). Log-rank testing did not reach statistical significance (p=0.058), though a trend toward shorter overall survival in monocytosis-positive patients was observed. Given the small sample size, group imbalance, and retrospective design, this finding should be interpreted as exploratory only.

Discussion

Pediatric central nervous system (CNS) tumors are the most common solid malignancies of childhood and encompass a biologically diverse spectrum.[16] Among these, low-grade gliomas—particularly pilocytic astrocytoma (PA)—represent roughly one-third of cases and generally have favorable outcomes due to their indolent behavior and amenability to resection.[16] In contrast, pediatric high-grade gliomas (pHGGs) account for only 8–12% of pediatric CNS tumors but remain among the most lethal, with long-term survival rates below 20% despite aggressive multimodal therapy.[16, 17]

Systemic Immune Dysregulation in Pediatric CNS Tumors

We previously reported pre-treatment lymphopenia in pediatric medulloblastoma, suggesting tumor-induced systemic immune suppression in childhood brain tumors.[12] While systemic immune dysregulation, particularly lymphopenia, has been recognized for decades in adult glioblastoma, analogous data in pediatric gliomas remain sparse.[18, 19] Here we sought to address this gap by characterizing pre-treatment hematologic immune profiles in children with H3K27M-mutant DMGs, using pilocytic astrocytoma as a biologically relevant, tumor-bearing comparator.

Prior studies have demonstrated that patients with posterior fossa tumors show elevated white blood cell counts, higher neutrophil-to-lymphocyte count ratios (NLCR), and increased mean platelet volume (MPV) relative to age- and sex-matched healthy controls.[20, 21] These shifts are thought to reflect a nonspecific inflammatory or stress response to the presence of a CNS neoplasm rather than tumor-specific biology. In contrast, CBC parameters in healthy pediatric cohorts, as defined by large reference datasets such as CALIPER, typically fall within narrow age- and sex-dependent ranges and do not display such elevations in the absence of pathology.[22]

Distinct Immune Signatures in H3K27M-Altered Gliomas

Contrary to our prior findings in medulloblastoma, we did not observe pre-treatment lymphopenia in the H3K27M cohort, suggesting that systemic lymphoid suppression is not a universal feature of pediatric brain tumors.[12] We did not detect a significant reduction in absolute lymphocyte counts among patients with H3K27M-mutant gliomas compared to those with PA, nor among patients with PA, indicating that the immune profiles of pediatric CNS tumors are heterogeneous and tumor-type specific. Similarly, we did not observe the peripheral neutrophilia commonly observed in adult glioblastoma.

However, we identified a consistent pattern of pre-treatment monocytosis in the H3K27M cohort, reflected by elevated AMC and MLR compared with PA controls. This immune signature may represent a distinct immunological phenotype of H3K27M-altered gliomas and demonstrates clear differences in circulating immune cell profiles between medulloblastoma and H3K27M midline gliomas, illustrating the importance of tumor-type–specific immune characterization.

It is important to contextualize these findings appropriately. The absolute differences in AMC and MLR between cohorts, while statistically significant, are modest in magnitude and most individual values remain within published pediatric reference ranges. These results are therefore best interpreted as reflecting a population-level shift in the myeloid compartment associated with H3K27M tumor biology, rather than as individual-level diagnostic markers. At the cohort level, this consistent myeloid signal suggests that H3K27M-mutant gliomas actively modulate systemic monocyte dynamics early in the disease course, a finding with potential implications for biomarker-driven risk stratification and immunotherapeutic targeting, pending prospective validation in larger, mutation-stratified cohorts.

An important interpretive caveat is that the current study design compares H3K27M-mutant high-grade gliomas against a low-grade tumor control. The observed monocytosis may in part reflect the systemic inflammatory response to high-grade tumor biology broadly, including tumor necrosis, mass effect, and cytokine release, rather than the H3K27M mutation specifically. Disentangling mutation-specific from grade-specific immune effects will require future studies incorporating H3K27M-wild-type pediatric high-grade gliomas, other pediatric diffuse glioma subtypes, and ideally paired tumor microenvironment data as additional comparator arms.

Biological Plausibility and Mechanistic Considerations

Peripheral monocytosis may represent tumor-driven immune modulation with monocytes and monocyte-derived cells being actively recruited into the tumor microenvironment, where they differentiate into tumor-associated macrophages (TAMs) that promote angiogenesis, immunosuppression, and resistance to therapy.[1-5, 23-25]This process is thought to be mediated in

part by tumor-derived cytokines and chemokines such as CCL2 and CSF1, which have been shown in preclinical glioma models to drive monocyte egress from bone marrow and peripheral blood into the tumor microenvironment.[26-28] Consistent with this, recent multiomic and spatial single-cell studies have shown that H3K27M gliomas are characterized by a myeloid-rich tumor microenvironment with abundant and heterogeneous macrophage populations, high immune checkpoint expression, and relative paucity of lymphoid cells.[29, 30]

Whether peripheral monocytosis directly reflects intratumoral macrophage burden, however, remains an open question. The relationship between peripheral immune cell counts and intratumoral immune composition is tumor-type dependent and not uniformly established.[31, 32] While correlations between circulating monocyte counts and macrophage infiltration have been described in some solid tumors, the intratumoral immune landscape in other brain tumors, such as medulloblastoma, appears governed primarily by local factors including vascular barrier integrity, MHC-I downregulation, and immunosuppressive cytokine signaling, rather than peripheral immune abundance.[33, 34] Peripheral monocytosis in H3K27M gliomas may therefore reflect a shared upstream driver promoting both systemic monocyte expansion and intratumoral macrophage recruitment in parallel, or may represent a partially dissociated systemic phenomenon. Direct correlation with paired tumor microenvironment profiling was not feasible in this retrospective cohort and is a priority for future investigation.

Clinical and Translational Implications

Our findings extend this paradigm to H3K27M-mutant pediatric diffuse midline gliomas, suggesting that monocytosis may serve as a unique biomarker of tumor-associated systemic

inflammation in H3K27M disease. While the mechanistic basis for this immune signature remains to be elucidated, these results emphasize the need to characterize tumor-type–specific immune alterations that may inform future immunotherapeutic strategies and risk stratification frameworks for children with high-grade gliomas.

By identifying potential biomarkers, we provide a focus for future translational and therapeutic research on pediatric high-grade gliomas harboring the histone H3K27M mutation. It should be noted, however, that the absolute numerical differences in AMC and MLR between cohorts are modest, and these metrics are best understood at present as population-level biological signals rather than individual-level clinical tests. The prognostic relevance of CBC-derived monocyte metrics has been established across multiple solid tumor types: elevated preoperative MLR has been associated with advanced stage and worse outcomes in ovarian and lung cancers, and elevated monocyte counts have been linked to systemic immunosuppression and tumor progression in cervical cancer.[35-38] Within neurooncology, NLCR has been consistently associated with poor prognosis in adult glioblastoma.[39] Our identification of a pre-treatment monocytic signature in pediatric H3K27M gliomas extends this framework to a distinct pediatric entity and provides a rationale for prospective investigation of MLR as a prognostic biomarker in this population. Consistent with this, an exploratory survival analysis in our cohort identified a trend toward shorter overall survival in monocytosis-positive patients, though this did not reach statistical significance given the limited sample size. Prospective studies incorporating both molecularly defined high- and low-grade glioma controls, as well as systematic survival outcome data, are needed to validate these findings and explore longitudinal changes during treatment.

Limitations

There are several limitations inherent to this study's retrospective design. Despite representing a significant molecularly defined pediatric H3K27M cohort, the modest sample size restricts granular subgroup analyses across tumor locations, ages, and outcomes. Additionally, while the use of pilocytic astrocytoma as a control group accounts for peri-diagnostic systemic stress, it may not entirely isolate H3K27M-specific biology from the broader differences between low- and high-grade CNS tumors. The observed monocytosis may in part reflect grade-related inflammatory biology rather than mutation-specific immune modulation, a distinction that future studies with H3K27M-wild-type high-grade glioma comparators will be important to address.

Furthermore, while corticosteroid use prior to CBC collection was an exclusion criterion, as corticosteroids can rapidly alter neutrophil and lymphocyte counts, the exact number of patients excluded on this basis was not prospectively recorded in a manner that permits precise reporting. Although this introduces a limitation in terms of reporting transparency, the exclusion criterion itself was applied systematically at both institutions uniformly, regardless of tumor location, age, or disease severity. Additional potential confounders including intercurrent infection, inflammatory comorbidities, and perioperative stress could not be fully evaluated in this retrospective dataset; the use of a tumor-bearing PA control group, subject to comparable peri-diagnostic stressors, partially mitigates this concern.

This study was intentionally limited to CBC-derived parameters available from routine clinical care and did not include PBMC subset profiling. Future studies employing flow cytometry or mass cytometry to characterize classical, intermediate, and non-classical monocyte populations will be essential to identify the specific monocyte subsets driving the observed systemic signal. Similarly,

the lack of longitudinal blood sampling prevents the assessment of immune profile evolution during treatment or its correlation with survival. Also, H3.1 (HIST1H3B/C) versus H3.3 (H3F3A) subtype information was not uniformly available across institutions, as mutation confirmation relied on a combination of immunohistochemistry, DNA methylation profiling, and sequencing. Whether the observed immune phenotype differs between H3.1 and H3.3 K27M variants is a question for future studies with comprehensive molecular annotation.

Conclusions

Pediatric H3K27M-mutant diffuse midline gliomas exhibit distinct systemic immune alterations characterized by pre-treatment monocytosis, suggesting a link between circulating myeloid activation and the tumor's immunosuppressive microenvironment. These findings have potential implications for immunotherapy development and biomarker-driven risk stratification in pediatric high-grade gliomas. Future studies integrating single-cell immune profiling and longitudinal blood–tumor immune correlations may further elucidate the biological and therapeutic significance of this myeloid signature.

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Conflict of Interest Statement

Matija Snuderl is a scientific advisor and shareholder of Heidelberg Epignostix and Halo Dx, a scientific advisor of Arima Genomics, and InnoSIGN, consulted for Servier Pharmaceuticals, and received research funding from Lilly USA. All other authors declare no competing interests.

Author Contributions:

Hannah Weiss: Conceptualization, Methodology, Investigation, Formal Analysis, Writing (original draft), Writing (review and editing). Eric A. Grin: Formal Analysis, Software, Visualization, Writing (original draft), Writing (review and editing), Project Administration. Anfal Alshammari: Resources, Writing (review and editing). Jonathan Serrano: Resources, Methodology, Validation, Writing (review and editing). Camilla Fang: Resources, Investigation, Writing (review and editing). Anirban Das: Resources, Investigation, Writing (review and editing). Cynthia Hawkins: Resources, Investigation, Writing (review and editing). Jessica Clymer: Resources, writing (review and editing). Devorah Segal: Resources, Writing (review and editing). Matthias A. Karajannis: Resources, Methodology, Writing (review and editing). David Harter: Resources, Writing (review and editing). Eveline Teresa Hidalgo: Resources, Methodology,

Supervision. Matija Snuderl: Conceptualization, Methodology, Resources, Validation, Funding Acquisition, Project Administration, Supervision,.

Ethics approval:

This study was approved by the NYU Langone Health Institutional Review Board (IRB# S14-00948). Patients' informed consent was obtained for all interventions, and the authors confirm that all research was conducted ethically in accordance with the principles outlined in the World Medical Association Declaration of Helsinki.

Data availability:

Data are available from the corresponding author upon reasonable request and with appropriate institutional permissions to protect patient privacy.

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Figure Captions:

Figure 1. Median CBC Metrics by Cohort. Pre-treatment peripheral blood counts in children with H3K27M-mutant diffuse midline glioma (blue, n=37) versus pilocytic astrocytoma (green, n=61). Panels show individual patient values and group distributions for **(A)** absolute neutrophil count (ANC), **(B)** absolute lymphocyte count (ALC), and (**C)** absolute monocyte count (AMC). Boxes represent the interquartile range (IQR) with the median shown as a white line; whiskers extend to 1.5×IQR; individual data points are overlaid. The dashed orange line indicates the CALIPER age-adjusted reference mean, which served as the threshold for defining monocytosis in this study. *p-values were calculated with the two-tailed Mann-Whitney U test; the asterisk indicates statistical significane at $p<0.05$.

Figure 2. Presence of Age-Adjusted Monocytosis. Proportion of children with age-adjusted monocytosis in the H3K27M diffuse midline glioma cohort versus the pilocytic astrocytoma cohort. Monocytosis was defined as an absolute monocyte count exceeding the published age-adjusted mean. Monocytosis was present in 78.4% of H3K27M cases (29/37) compared with 46.7% of PA cases (28/61), corresponding to an odds ratio of 4.14 (p=0.003).

Children with an aggressive brain tumor called H3K27M-mutant glioma face limited treatment options. This study looked at blood tests taken before treatment began. Researchers discovered that these patients have higher levels of a specific white blood cell, called a monocyte, compared to children with less aggressive tumors. This finding suggests that these tumors trigger a unique immune response in the body. Understanding this "immune signature" is a vital step toward finding new ways to monitor the disease and developing future therapies that help the immune system fight these specific childhood brain tumors.

Table 1. Study results. SD standard deviation, ANC absolute neutrophil count, ALC absolute lymphocyte count, AMC absolute monocyte count, NLCR neutrophil-to-lymphocyte count ratio, MLR monocyte-to-lymphocyte count ratio

	H3K27M (n=37)		**PA (n=61)**		***p***
Sex	19 female, 18 male		28 female, 33 male		
Median age in years [IQR] (range)	8 [4-11] (2 – 17)		6 [3-10] (0.25-18)		0.177
	Mean ± SD	*Median*	*Mean ± SD*	*Median*	
ANC [K/µL]	4.49 ± 3.85	4.49	4.44 ± 3.06	4.44	0.800
ALC [K/µL]	2.70 ± 1.71	2.70	2.88 ± 1.79	2.88	0.621
AMC [K/µL]	0.70 ± 0.32	0.70	0.50 ± 0.30	0.50	0.012*
NLCR	1.35 ± 1.90	1.35	1.31 ± 3.69	1.31	0.550
MLR	0.20 ± 0.17	0.20	0.19 ± 0.16	0.19	0.021*
Monocytosis (%)	n = 29 (78.4)		n = 28 (46.7)		0.003*

*statistically significant at $p<0.05$

Figure 1

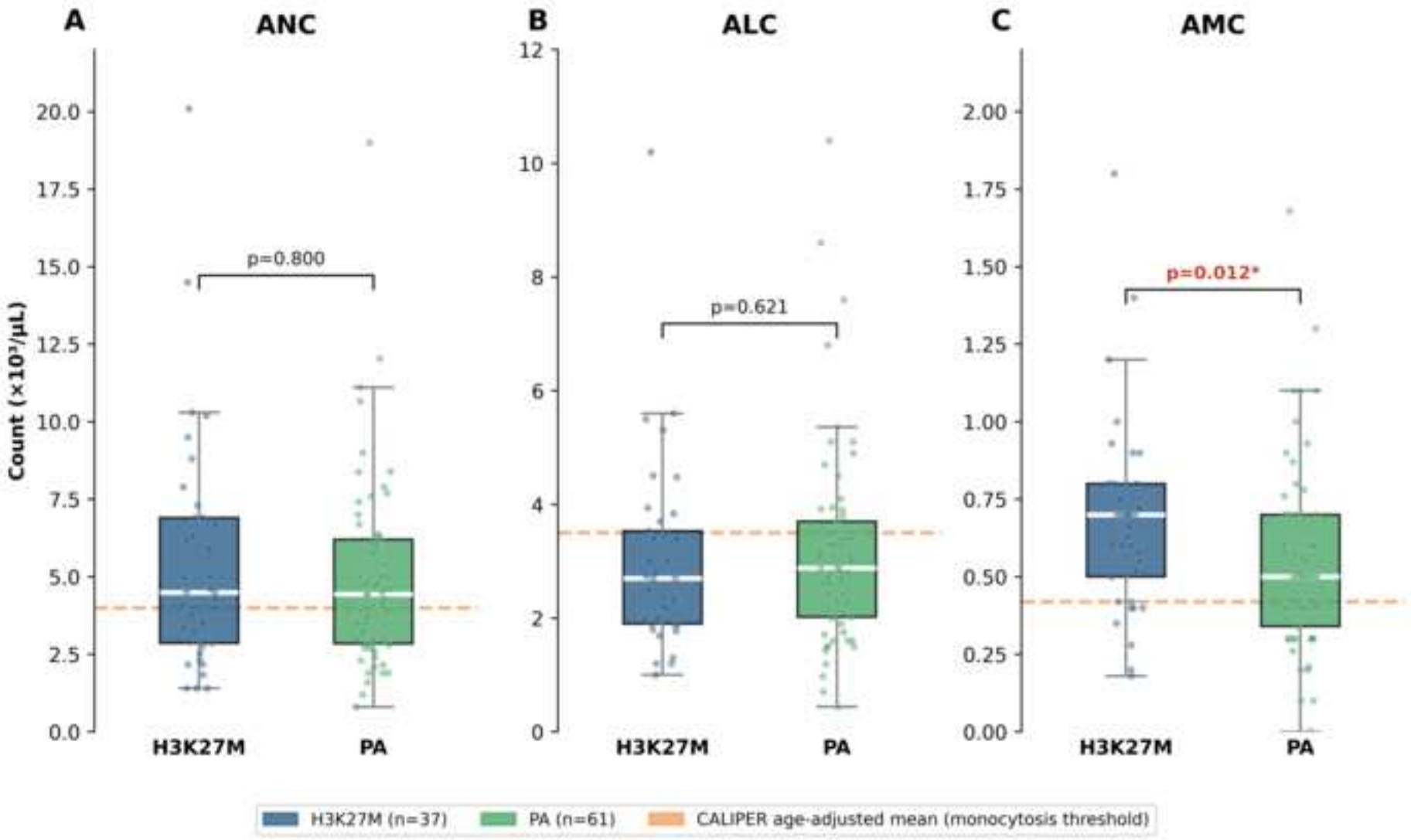

Figure 2