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Microsurgical brainstem biopsy through safe entry zones for children with radiologically suspected diffuse intrinsic pontine glioma: a single-center experience from Brazil

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Abstract

Purpose.

Diffuse intrinsic pontine glioma (DIPG) was historically diagnosed by imaging alone, but current classification has renewed the role of brainstem biopsy. Most published series are stereotactic, and data from Latin America are limited. We report the safety, yield, and utility of microsurgical brainstem biopsy through safe entry zones in children with radiologically suspected DIPG at a Brazilian center.

Methods.

Retrospective single-center cohort of consecutive patients aged 19 years or younger who underwent open microsurgical brainstem biopsy through a safe entry zone between April 2018 and December 2024. Clinical, surgical, molecular, and survival data were collected. Overall survival was estimated using Kaplan-Meier analysis, from biopsy to death or last follow-up.

Results.

Nineteen patients were included (median age, 60 months). Entry zones were lateral pontine in 57.9%, lateral bulbar in 26.3%, and supratrigeminal in 15.8%. Tissue yield was 100%, with no mortality or permanent neurological deficit; one patient (5.3%) had transient mutism. Integrated pathology molecular diagnoses were diffuse midline glioma, H3 K27-altered in 12 patients (63.2%; 10 H3F3A and 2 HIST1H3B K27M); molecularly unconfirmed DIPG in 5 (26.3%); pilocytic astrocytoma in 1 (5.3%); and embryonal tumor with multilayered rosettes in 1 (5.3%). Median overall survival was 14.0 months, and 10.0 months in the H3 K27-altered subgroup. Biopsy changed management or enabled trial enrollment in 3 patients (15.8%).

Conclusion.

Open microsurgical brainstem biopsy provided high tissue yield, low morbidity, and clinically meaningful stratification, supporting selective implementation in referral centers with microsurgical and histopathology expertise.

Introduction

Pediatric brainstem tumors account for approximately 10–20% of pediatric central nervous system neoplasms, and diffuse intrinsic pontine glioma (DIPG) accounts for about 80% of these cases [2, 30]. The prognosis for DIPG remains poor, with median overall survival ranging from 9 to 12 months and 2-year survival rates to date below 10%, as reported in most contemporary studies [15, 42]. For nearly three decades, the diagnosis of DIPG was based solely on magnetic resonance imaging (MRI) findings, following the influential 1993 report by the Children's Cancer Group, which argued that biopsy did not increase diagnostic accuracy or provide additional therapeutic guidance, while exposing children to unnecessary risks [1].

This prognosis has been redefined by molecular discoveries. The identification in 2012 of recurrent K27M mutations in the tail of histone H3 redefined DIPG as a fundamentally epigenetic disease [17, 35, 46], a biological insight that the 2021 World Health Organization classification formalizes as a distinct entity: diffuse midline glioma, H3 K27-altered [22]. This category aggregates tumors that converge on a common epigenetic phenotype through different molecular

pathways, most commonly an H3K27M mutation in non-canonical histone H3 3 (encoded by H3F3A) or in canonical histone H3.1 (HIST1H3B/C), but also EZHIP overexpression or EGFR alteration in wild-type H3 tumors. Since these entities are radiologically indistinguishable (to date) but differ in terms of biology, accurate diagnosis increasingly depends on tissue-based molecular characterization rather than imaging alone [8].

Advances in image-guided stereotactic techniques have been reported in pediatric series demonstrating the feasibility of biopsying pontine lesions [33]. Recent studies and meta-analyses indicate that brainstem biopsy, performed with meticulous navigation informed by a deep understanding of brainstem neuroanatomy, can achieve a diagnostic yield exceeding 95% and permanent morbidity rates below 2% in experienced centers [12, 13, 27, 37, 38]. These results challenge the initial arguments against biopsy based on patient risk and have led to a proactive approach to stereotactic biopsy as an important part of routine care for newly diagnosed DIPG [45]. Despite these advances, some knowledge gaps remain. Most contemporary studies describe stereotactic techniques, whether or not robot-assisted. A recent meta-analysis evaluating 1,002 pediatric brainstem biopsies revealed that 87% were performed stereotactically, while only 12.7% were performed via open surgery [37]. Data on open microsurgical biopsy through safe entry zones in the brainstem (anatomical and functional areas that provide access to the pons with lower risk to critical structures) remain limited in the era of DIPG. Another point is that Latin America is underrepresented in the literature; only one previous Brazilian series has been published, which included a smaller sample, used a stereotactic approach, and did not perform systematic molecular analysis [25]. Third, in healthcare settings without local access to targeted therapy trials, the role of biopsy is unclear; and this may be an issue of implementation: while high-income countries commonly justify biopsy for inclusion in clinical trials, this justification generally does not apply to most Brazilian patients. It has not yet been formally evaluated how a safe and reproducible diagnostic workflow can be implemented in centers without local robotic platforms or that lack biomarker-based clinical trials, nor which children benefit even when no immediate targeted therapy is available.

Our objective was to evaluate whether performing microsurgical biopsies using safe, established access zones, within a multidisciplinary workflow, can facilitate reliable diagnostic clarification and clinically meaningful stratification of pediatric patients with radiologically suspected DIPG at a Latin American referral center. We report a consecutive series of 19 pediatric patients with radiologically suspected DIPG who underwent microsurgical brainstem biopsy via a safe entry zone between 2018 and 2024 at the Children's Cancer Hospital, Barretos, São Paulo, Brazil. We describe the safety profile, tissue yield, histopathology, molecular distribution, and survival outcomes of this cohort, and discuss the value of implementing brainstem biopsy in Latin American healthcare settings.

Methods

Study design and patient selection

This is a retrospective observational cohort study of all consecutive pediatric patients who underwent open microsurgical biopsy of the brainstem via a safe access zone at the Children's Cancer Hospital in Barretos, São Paulo, Brazil, between April 2018 and December 2024. The inclusion criteria were: (i) age ≤ 19 years at the time of biopsy; (ii) an expansive intra-axial brainstem lesion with its epicenter in the pons on preoperative MRI; and (iii) imaging features consistent with typical DIPG (diffuse pontine expansion, T1 hypointense signal, T2/FLAIR hyperintense signal, absent or minimal contrast enhancement) or with atypical features, such as heterogeneous post-contrast enhancement or the presence of an exophytic component. The exclusion criteria were: (i) lesions with an epicenter located outside the pons (midbrain or medulla oblongata); (ii) lesions with radiological features suggestive of a vascular malformation, including cavernous malformation. The study was approved by the institution's research ethics committee (approval number: 78910824.6.1001.5437).

Preoperative evaluation

All patients underwent gadolinium-enhanced magnetic resonance imaging, including diffusion-weighted, T1, T2, FLAIR, post-gadolinium T1, and tractography sequences. The images were reviewed by a neuroradiologist, pediatric neurosurgeons, and the pediatric oncology team. The biopsy target and entry site were selected through a multidisciplinary review of the preoperative MRI and neurological findings on physical examination. The primary criterion was the safest surgical approach through an established brainstem access route. When more than one viable approach was available, the target was preferentially directed to regions expected to maximize diagnostic yield, including exophytic tumor components, areas of contrast enhancement, or regions of diffusion restriction. When anatomically appropriate, the approach was also preferably planned on the side of a preexisting neurological deficit in order to minimize the risk of additional clinically significant morbidity.

The decision to proceed with the biopsy was made on a case-by-case basis, following team discussion and obtaining informed consent from the family, after a structured discussion regarding the rationale for the procedure, the risks, and the expected yield of information.

Surgical technique

All procedures were performed under general anesthesia, with patients in the “park bench” position. We performed a retrosigmoid craniotomy, and the access side was selected based on the possibility of the most direct path to the lesion or target area. Neuronavigation (Brainlab®) was registered based on preoperative volumetric magnetic resonance imaging. Intraoperative neurophysiological monitoring included somatosensory evoked potentials, motor evoked potentials, electroencephalogram with spectral edge frequency, continuous electromyography, and stimulated electromyography using a monopolar probe with single-pulse techniques. The dura mater was opened, cerebrospinal fluid was drained from the cerebellomedullary cistern, and the cerebellum was gently retracted to expose the cerebellopontine and cerebellomedullary angles. Dynamic retraction using a suction device was preferred over static retractors to minimize cerebellar injury. The relevant cranial nerves were identified, and the brainstem was approached through one of the safe entry zones [5] according to tumor topography: (i) *the supratrigeminal zone*, located just above the trigeminal root entry zone on the middle cerebellar peduncle, to approach rostral pontine lesions with projection toward the pontomesencephalic junction; (ii) *the lateral pontine zone*, a safe corridor on the junction between the middle cerebellar peduncle and the pons and between the trigeminal and the facial-vestibulocochlear complex root entry zones, used for lesions centered in the pons with lateral extension; (iii) *the lateral bulbar area*, a relatively safe entry zone used for pontine lesions with dorsolateral extension to the medulla, accessed through a small vertical incision in the inferior cerebellar peduncle, below the cochlear nuclei and posterior to the origin of the glossopharyngeal (IX) and vagus (X) cranial nerves.

In all cases, a small pia mater incision and microsurgical dissection were performed. Following neurophysiological stimulation, tissue samples were obtained with minimal use of bipolar coagulation. Multiple samples (typically six to eight) were collected to ensure sufficient material for histopathology (formalin-fixed, paraffin-embedded [FFPE] sections) and molecular analysis (fresh-frozen tissue or tissue preserved in RNAlater), as well as for translational research platforms using patient-derived xenograft (PDX) models for pediatric brain tumors currently being developed at our institution [40]. We confirmed hemostasis with continuous irrigation before closing the dura mater and repositioning the bone flap with mini-plates and mini-screws. After the procedure, all patients were transferred to the intensive care unit under ambient air conditions.

Histopathological and molecular analysis

All samples were analyzed by a specialist neuropathologist. Routine immunohistochemistry included GFAP, OLIG2, ATRX, p53, Ki-67, IDH1-R132H, and, when available, H3K27me3 (clone C36B11).

Molecular analysis evolved over the course of the study, depending on the availability of institutional and national resources. Between 2018 and 2020, Sanger sequencing was performed on exon 2 of H3F3A (codons 28 and 35) and codon 28 of HIST1H3B, as reported by Castel et al [4]. Starting in 2021, a next-generation sequencing assay was used, based on an institutional panel previously established in the Molecular Diagnostics Laboratory at Barretos Cancer Hospital [26] covering BRAF V600, IDH1, IDH2, H3F3A, HIST1H3B, TERT promoter, and FGFR1 exons 12–14. Selected cases from 2024 onward underwent additional testing using an RNA-based fusion panel (Sophia Genetics, Saint-Sulpice, Switzerland), targeting ALK, EGFR, FGFR1–4, MET, NTRK1–3, RET, ROS1, and others. BRAF V600E mutational status (by RT-PCR) and KIAA1549-BRAF fusion (by FISH) were assessed in selected cases. C19MC amplification was evaluated by FISH in lesions with embryonal histology. EZHIP immunohistochemistry and whole-genome DNA methylation profiling were not performed systematically during the study period.

For this analysis, tumors were classified according to the WHO Classification of Tumors of the Central Nervous System, 5th edition (CNS5), as follows: diffuse midline glioma, H3 K27-altered, was assigned when a K27M variant of H3F3A or HIST1H3B was detected by sequencing, and nuclear trimethylation of H3K27me3 was absent on immunohistochemistry, in the context of a diffuse glioma morphology. Cases that met the clinical and radiological criteria for DIPG but lacked molecular confirmation were classified as molecularly unconfirmed DIPG when sequencing was negative for H3F3A and HIST1H3B, and no additional confirmatory immunohistochemistry was available. Tumors meeting integrated histopathology and molecular criteria for another WHO entity (e.g., pilocytic astrocytoma or embryonal tumor with multilayered rosettes and C19MC alteration) were classified accordingly.

Outcome definitions

Tissue yield (diagnostic) was defined as the proportion of procedures that yielded sufficient tissue for histopathological diagnosis and for at least one molecular test. Molecular yield was defined separately as the proportion of cases in which an integrated WHO CNS5 molecular entity could be assigned. Postoperative complications were classified as transient (complete resolution within 30 days) or permanent (persistent deficit for more than 30 days) and graded according to the Common Terminology Criteria for Adverse Events, version 5.0 [28]. Overall survival was calculated from the date of biopsy at our institution until death from any cause, with the data cutoff date for the survival analysis being May 18, 2026. Patients who were alive on that date were censored on the cutoff date; patients lost to follow-up prior to that date were censored on the date of their last documented clinical contact. Postoperative length of stay (LOS) was defined as the interval, in calendar days, between the date of surgery and the date of hospital discharge for outpatient follow-up; readmissions for subsequent oncological care were not considered.

Statistical analysis

Continuous variables were summarized as medians with interquartile ranges (IQRs), and ranges were reported when appropriate. Categorical variables were summarized as absolute numbers and percentages. Overall survival was defined as the interval from biopsy to death from any cause or last follow-up and was estimated for the entire cohort using the Kaplan-Meier method. The median overall survival was defined as the earliest time point at which the

Kaplan-Meier survival estimate was ≤ 0.50. Because of the small number of patients within each histomolecular subgroup, subgroup-level survival was displayed descriptively using a swimmer plot rather than stratified Kaplan-Meier curves or formal between-group statistical comparisons. Survival estimates were calculated using a custom Python implementation of Kaplan-Meier based on NumPy, and figures were generated with Matplotlib.

Results

Patient characteristics

Nineteen patients underwent microsurgical brainstem biopsy through a safe entry zone between April 2018 and December 2024. Twelve patients (63.2%) were female, and seven (36.8%) were male, resulting in a female-to-male ratio of 1.7:1. The median age at the time of biopsy was 60 months (5.0 years), with an interquartile range of 48 to 114 months (4.0 to 9.5 years) and a range of 24 to 228 months (2 to 19 years). All patients met the radiological inclusion criteria for DIPG. Clinical presentations included cranial nerve palsies, signs of long-tract involvement, ataxia, and, in some cases, signs of increased intracranial pressure. Detailed individual data are presented in **Table 1**.

Surgical approach and operative course

All 19 procedures were performed through a retrosigmoid craniotomy under microsurgical visualization. The lateral pontine entry zone was used in 11 patients (57.9%), the lateral bulbar area in 5 patients (26.3%), and the supratrigeminal zone in 3 patients (15.8%). No procedure required conversion to an alternative approach. There were no intraoperative cardiopulmonary events, and no patient required an intraoperative blood transfusion. Postoperative length of hospital stay was available for all 19 patients. Excluding one patient with non-surgical confounding factors (Patient 15; see the footnote in **Table 2**), the median length of hospital stay was 4.5 days (interquartile range 3–7; range 1–15), and 14 of the 18 patients (77.8%) were discharged within seven days after the procedure. Patient 15 had a hospital stay of 35 days due to circumstances unrelated to post-biopsy recovery: ten days before the operative intervention, the patient was transferred to our facility while intubated for a significantly decreased level of consciousness, underwent urgent placement of a ventriculoperitoneal shunt, and continued inpatient care in order to initiate radiation therapy on the 7th postoperative day.

Safety profile, tissue yield, histopathology and molecular profile

No patient died as a result of the biopsy, and no new permanent neurological deficits were attributable to the procedure. One patient (Patient 18; 1 of 19, 5.3%) developed transient postoperative mutism that resolved completely over the course of 16 days without specific treatment; this patient was diagnosed with ETMR and remains alive and disease-free at the last follow-up. Eighteen patients (94.7%) had no postoperative neurological complications. There were no postoperative hematomas, cerebrospinal fluid leakages, or infections at the surgical site. Tissue yield rate was 19/19 (100%): all biopsies provided sufficient tissue for both integrated histopathological evaluation and at least one targeted molecular test. An integrated WHO CNS5 diagnosis was established in 14 of the 19 cases (73.7%); the remaining five (26.3%) met the clinical and radiological criteria for DIPG but lacked the molecular confirmation required for an H3 K27-altered diagnosis and were classified as molecularly unconfirmed DIPG.

Integrated histological and molecular diagnoses were as follows: diffuse midline glioma, H3 K27-altered, in 12 patients (63.2%); molecularly unconfirmed DIPG in 5 patients (26.3%); pilocytic astrocytoma in 1 patient (5.3%); and embryonal tumor with multilayered rosettes (ETMR) with C19MC amplification in 1 patient (5.3%) (**Fig. 1**). Among the

twelve cases with H3 K27 alterations, an H3F3A K27M variant (**Fig. 2**) was detected in 10 patients (83.3%), and a HIST1H3B K27M variant (**Fig. 3**) in 2 (16.7%); all were confirmed by Sanger or next-generation sequencing.

Five molecularly unconfirmed DIPG cases included three patients (patients 7, 10, and 16) with loss of nuclear H3K27me3 expression on immunohistochemistry but lacking the molecular or methylation tests required for confirmation; one patient (patient 13), who was wild-type for both IDH and H3 on integrated testing; and one patient (patient 19), who had negative results for H3F3A and HIST1H3B on both Sanger and NGS hotspot sequencing,

without confirmatory immunohistochemistry.

In none of these five cases were the CNS5 essential criteria for an H3 K27-altered diagnosis met: immunohistochemical H3K27me3 loss alone is insufficient, and EZHIP immunohistochemistry and DNA methylation profiling were not systematically performed during the study period.

Survival analysis

Median follow-up from biopsy was 14.0 months (IQR 8.9–20.1). Sixteen of nineteen patients died during follow-up, and three remained alive at the data cutoff (Patients 17 and 18, with non-DMG diagnoses, and Patient 19, molecularly unconfirmed DIPG, alive at 96.7 months). Median overall survival in the entire cohort was 14.0 months, with 12-month and 24-month overall survival estimates of 68.4% and 21.1%, respectively (**Fig. 4a**).

Individual overall survival by histomolecular subgroup is shown as a swimmer plot (**Fig. 4b**). In the H3 K27-altered subgroup (n = 12), median overall survival was 10.0 months, with 12-month and 24-month survival of 50.0% and 0%, respectively. Among the five molecularly unconfirmed DIPG patients, four died (at 13.0, 14.0, 19.8, and 26.3 months) and one (patient 19) remained alive at 96.7 months. The two patients with other diagnoses (pilocytic astrocytoma and ETMR) remained alive at last follow-up (30.7 and 49.6 months, respectively). Given the small subgroup sizes, no formal between-group comparison was performed.

Among the five molecularly unconfirmed DIPG cases, one (Patient 19, **Fig. 5**) deserves separate consideration. This 3-year-old female presented in April 2018 with a radiographically classic pontine lesion, underwent microsurgical biopsy through the right lateral bulbar entry zone, and was found to harbor no detectable variants in H3F3A, HIST1H3B, BRAF, IDH1/2, TERT, or FGFR1 on combined NGS and Sanger sequencing. Following 54 Gy of focal radiotherapy, the patient was enrolled in the CheckMate 908 trial and treated with nivolumab plus ipilimumab [10]. At last follow-up (May 2026), the patient remains alive with stable residual disease 96.7 months after biopsy — a survival pattern highly unusual in the H3 K27-altered DMG literature, although rare long-term survivors with co-occurring MAPK pathway alterations have been described in H3K27M-mutant cohorts [32].

Table 1. Individual characteristics, treatment and survival of the 19 patients.

Pt	Sex	Age (months)	Year of biopsy at our center	Safe entry zone	Histomolecular diagnosis	Adjuvant treatment (summary)	OS (m)	Status
1	F	60	2024	Left lateral pontine	DMG, H3K27-altered (HIST1H3B K27M, NGS)	RT (4 sessions only)	2.6	Deceased (3/2025)
2	M	84	2018	Right supratrigeminal	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 54 Gy	5.4	Deceased (8/2018)
3	F	36	2020	Right lateral pontine	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 39 Gy (hypofractionated)	6.5	Deceased (7/2021)
4	M	48	2023	Left lateral bulbar	DMG, H3K27-altered (H3F3A K27M, NGS)	RT 39 Gy (hypofractionated)	7.0	Deceased (5/2024)
5	F	84	2024	Right supratrigeminal	DMG, H3K27-altered (H3F3A K27M, NGS)	RT 54 Gy + TMZ (interrupted)	7.8	Deceased (3/2025)
6	F	108	2018	Right lateral pontine	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 59.4 Gy	10.0	Deceased (10/2019)
7	F	48	2023	Right lateral bulbar	Molecularly unconfirmed DIPG (H3K27me3 IHC loss; molecular profiling unavailable)	RT 54 Gy + re-RT 36 Gy	13.0	Deceased (5/2024)
8	F	36	2023	Right lateral bulbar	DMG, H3K27-altered (H3F3A K27M, NGS)	RT 39 Gy (hypofractionated)	13.5	Deceased (8/2024)
9	M	120	2023	Left lateral pontine	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 54 Gy + re-RT 36 Gy	13.8	Deceased (5/2024)
10	M	24	2023	Right lateral pontine	Molecularly unconfirmed DIPG (H3K27me3 IHC loss; molecular profiling unavailable)	RT 54 Gy + re-RT 30 Gy	14.0	Deceased (11/2024)
11	F	120	2020	Left lateral pontine	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 54 Gy + re-RT 20 Gy	15.4	Deceased (10/2021)
12	M	144	2019	Right lateral pontine	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 54 Gy	17.3	Deceased (11/2020)

Pt	Sex	Age (months)	Year of biopsy at our center	Safe entry zone	Histomolecular diagnosis	Adjuvant treatment (summary)	OS (m)	Status
13	F	228	2023	Left lateral pontine	Molecularly unconfirmed DIPG (IDH-wildtype, H3-wildtype)	Prior RT (2022) elsewhere; TMZ; partial resection ×2; re-RT + bevacizumab	19.8	Deceased (8/2024)
14	F	168	2023	Right lateral pontine	DMG, H3K27-altered (H3F3A K27M, Sanger)	RT 54 Gy + re-RT 36 Gy + SRS + bevacizumab	20.0	Deceased (12/2024)
15	F	48	2023	Left lateral pontine	DMG, H3K27-altered (HIST1H3B K27M, NGS)	RT 39 Gy + re-RT 36 Gy	20.2	Deceased (11/2024)
16	M	60	2024	Right supratrigeminal	Molecularly unconfirmed DIPG (H3K27me3 IHC loss; molecular profiling unavailable)	RT 54 Gy	26.3	Deceased (3/2026)
17[a]	F	96	2023	Left lateral bulbar	Pilocytic astrocytoma (BRAF V600E neg; KIAA1549-BRAF fusion absent)	Prior CTx (2019–21) elsewhere; biopsy + RT (2023)	30.7	Alive, stable residual disease
18	M	48	2022	Right lateral pontine	ETMR, C19MC-amplified (FISH)	CSI + Jakacki protocol; transient mutism (16 d)	49.6	Alive, disease-free
19	F	36	2018	Right lateral bulbar	Molecularly unconfirmed DIPG (NGS + Sanger neg)	RT 54 Gy → trial CA209-908 (nivolumab + ipilimumab, ongoing)	96.7	Alive, stable residual disease

Abbreviations: DMG, diffuse midline glioma; DIPG, diffuse intrinsic pontine glioma; ETMR, embryonal tumor with multilayered rosettes; F, female; LOS,

postoperative length of stay (days, between surgery and hospital discharge); M, male; NGS, next-generation sequencing; OS, overall survival from biopsy;

RT, radiotherapy; SRS, stereotactic radiosurgery; TMZ, temozolomide. [a]Patient 17 had been treated empirically as radiologically suspected DIPG at another

Institution in 2019 (chemotherapy, without biopsy); the first histological diagnosis (pilocytic astrocytoma) was established by biopsy at our center in 2023,

from which OS is calculated.

Table 2. Postoperative length of stay (days)

Pt	LOS (days)	Pt	LOS (days)
1	2	11	3
2	6	12	5
3	3	13	1
4	14	14	8
5	4	15	35[a]
6	2	16	3
7	9	17	3
8	7	18	15
9	5	19	6
10	2	—	—

LOS, postoperative length of stay, calculated as the interval in calendar days between the date of the biopsy and the date of hospital discharge to outpatient follow-up. [a]Patient 15 was admitted intubated with depressed consciousness ten days before biopsy, underwent emergency ventriculoperitoneal shunting before the biopsy, and remained hospitalised for the initiation of radiotherapy seven days after the procedure; her length of stay therefore does not reflect post-biopsy recovery alone. Median LOS for the remaining 18 patients was 4.5 days (interquartile range 3–7; range 1–15).

Table 3. Diagnostic yield and complications across published pediatric brainstem biopsy series. Data for the comparator series were compiled from the original published reports [2, 7, 12, 13, 21, 25, 27, 31, 37, 47].

Series	n	Approach	Diagnostic yield	Transient morbidity	Permanent morbidity	Mortality
Hamisch 2017 (meta-analysis) [13]	735	Stereotactic (frame/frameless)	96.1%	6.7%	0.6%	0.6%
Gupta (DIPG-BATS) 2018 [12]	50	Stereotactic transcerebellar	100%	NR	2.0%	0%
Puget 2015 (single-center) [31]	130	Stereotactic	100%	3.9%	0%	0%
Mueller (Zurich) 2023 [27]	25	Robot-assisted stereotactic	96%	8%	0%	0%
Morais (USP, Brazil) 2020 [25]	26	Stereotactic (76.9% transcerebellar)	84.6%	7.7%	0%	0%
Boukaka (Lyon) 2023 [2]	48	Mixed (open + stereotactic)	NR	29% (incl. transient)	NR	2.2%
Sheikh 2024 (meta-analysis) [37]	1002	Stereotactic (87%) / open	96.8%	6%	1%	0.5%
Dalmage 2023 (SR, DIPG-only) [7]	192	Stereotactic	97.4%	13.0% (any)	NR (not separated)	0%
Zhao 2025 (robotic meta-analysis) [47]	215	Robotic (frame/frameless)	98%	6%	0.9% (2/215)	0%
Lim (Singapore) 2022 [21]	21	Mixed (open + stereotactic)	100%	NR	0%	0%
Present series (Barretos)	19	Microsurgical, via SEZ	100%	5.3%	0%	0%

Abbreviations: NR, not reported; SEZ, safe entry zone. Definitions of “transient” and “permanent” morbidity vary across studies. Direct

comparison should be made with caution because morbidity categories and study populations are not uniform across series.

Discussion

This study describes the experience of a single Brazilian referral center with microsurgical brainstem biopsy using safe access zones in 19 children with radiologically suspected DIPG between 2018 and 2024. What we report here is not the use of expensive surgical technology, but rather the implementation of a reproducible, expertise-driven microsurgical protocol, combined with contemporary histopathological and molecular diagnostics, within a healthcare system where access to clinical trials involving targeted therapies remains quite limited. The procedure achieved a 100% tissue yield with a safety profile (0% mortality, 0% permanent morbidity, 5.3% transient morbidity), a result consistent with the upper limit of the largest stereotactic series in the literature (**Table 3**).

Of the 19 patients with radiologically suspected DIPG, 12 (63.2%) met the current essential WHO criteria for DMG, H3 K27-altered, while the remaining seven comprised five molecularly unconfirmed pons gliomas—one of whom survived for nearly eight years on immunotherapy—and two non-DIPG entities with markedly different prognoses and

management (pilocytic astrocytoma and ETMR). Even in the absence of targeted therapy clinical trials available in our country, the biopsy refined the diagnosis or enabled inclusion in a clinical trial. Biopsy had immediate implications for management in at least 3 of the 19 patients (15.8%): two alternative diagnoses (pilocytic astrocytoma and ETMR) and one case that was not molecularly confirmed but was included in a clinical trial (Patient 19).
Contemporary meta-analyses of pediatric brainstem biopsies report a combined diagnostic yield of 96–97%, transient morbidity of 6–10%, permanent morbidity of approximately 1%, and procedure-related mortality of 0.5–0.6% [13, 37]. The prospective DIPG-BATS study achieved a 100% diagnostic yield, with a single case of permanent hemiparesis among 50 stereotactic biopsies [12]. The Zurich experience with robot-assisted biopsy reported a 96% success rate, with no permanent morbidity in 25 cases [27]. The largest single-center series of stereotactic biopsies in DIPG (Puget et al., n = 130) reported a 100% success rate and a transient morbidity rate of 3.9% [31]. Dalmage et al. published a systematic review of stereotactic biopsy for pediatric DIPG (13 studies, 192 patients), reporting a diagnostic yield of 97.4% and a neurosurgical complication rate of 13.0%, with no procedure-related mortality [7]. The aggregate number of complications includes all events attributed to the biopsy and does not distinguish between transient and permanent deficits; therefore, it is not directly comparable to our results of 5.3% transient deficits and 0% permanent deficits. In this context, the present series—with a 100% success rate, 5.3% transient morbidity, and no permanent morbidity or mortality—is consistent with the upper limit of published safety performance, despite using an open microsurgical approach that has become uncommon in contemporary practice (12.7% of all pediatric brainstem biopsies in the meta-analysis by Sheikh et al.) [37]. This safety profile is comparable to that achieved by technologies that require greater resources. A recent single-arm meta-analysis of robot-assisted stereotactic biopsy in 215 children reported a 99% technical success rate and a 98% conclusive diagnostic yield, with transient complications in 6% and permanent morbidity in only two patients, and no procedure-related mortality [47]. Single-center series of frameless robotic-assisted biopsy also reported high success rates with low rates of permanent morbidity [16, 29]. The fact that an open microsurgical approach can match the diagnostic accuracy and safety of these robotic platforms is significant from the perspective of global neurosurgery and global health: in centers with established microsurgical expertise and a multidisciplinary approach, it is possible to achieve equivalent diagnostic and translational benefits, provided there is surgical expertise and an integrated molecular approach.

Several features of the open microsurgical approach are worth mentioning. Direct microscopic visualization of the brainstem surface allows for precise identification of the cranial nerves, blood vessels, and any exophytic tumor components; tissue sampling is targeted and can be repeated at different topographic locations, increasing the likelihood of obtaining representative material. The amount of tissue obtained is generally greater than with a stereotactic needle, which is relevant for parallel workflows in histopathology, immunohistochemistry, and molecular analysis, as well as for tissue banks and patient-derived xenograft (PDX) model platforms. Hemostasis can be performed under direct visualization if bleeding occurs, unlike in the stereotactic procedure. These conceptual advantages are not unique to our series; they have been emphasized in canonical descriptions by Kyoshima [20] and Cavalheiro [6], and served as the foundation for the historic Brazilian school of brainstem surgery. The steep learning curve associated with this approach and the need for a microneurosurgery suite equipped with a neuronavigator and neurophysiological monitoring may limit its widespread adoption beyond specialized centers. In the present series, the choice of entry site was individualized according to the tumor's topography. This anatomy-guided selection, rather than a single fixed corridor, is essential for reducing the risk of injury to the cranial nerve nuclei and long tracts.
Postoperative hospital stay is a clinically significant indicator, but has been reported inconsistently in a series of brainstem biopsies in children. Among the largest published cohorts, only the robot-assisted series from Zurich provides explicit summary statistics (median of 2 days, range 1 to 4) [27]. The majority of recent series, including the prospective DIPG-BATS study (which reports only a median interval of 10 days [range 4–22] between surgery and initiation of radiotherapy) [12], do not report length of hospital stay as a discrete outcome. Similarly, this parameter is

not addressed in the meta-analyses by Hamisch et al. [13] and Sheikh et al. [37], nor in the systematic review by Dalmage et al. [7]. In our cohort, the median length of hospital stay was 4.5 days (IQR 3–7; range 1–15), with 77.8% of patients being discharged within seven days. The slightly longer hospital stay compared with the Zurich stereotactic experience is consistent with the somewhat more invasive nature of the retrosigmoid microsurgical approach and with the institutional discharge practices of a referral center serving patients from across Brazil, where immediate discharge is often impractical given the logistics of remote follow-up. The only significantly atypical case (Patient 15, 35 days) had a longer hospital stay that was determined by clinical events prior to surgery—the child was admitted from another institution already on mechanical ventilation, and after the procedure, the patient remained hospitalized to begin radiation therapy during the hospital stay, rather than by post-biopsy recovery. Although the size of our sample precludes definitive conclusions, the data contribute to the literature on brainstem biopsy in pediatric patients and suggest that open microsurgical biopsy via safe access sites can be performed without substantially increasing hospital LOS.

Seven of the nineteen patients (36.8%) with suspected DIPG on MRI did not have a molecularly confirmed H3 K27 mutation in the available tests. The cohort included both lesions with the classic DIPG appearance on imaging and lesions with atypical features (heterogeneous, intense, or irregular post-contrast enhancement, or an exophytic component); this design reflects the actual spectrum of suspected pons gliomas referred to our service, rather than a selected population of radiologically prototypical cases. The observed proportion of non-canonical or unconfirmed diagnoses is consistent with previous reports that question the specificity of the radiological diagnosis: Schumacher et al., in a multicenter review of 142 children with brainstem disease, reported a sensitivity of 94%, but a specificity of only 43% for magnetic resonance imaging (MRI) in distinguishing between tumorous and non-tumorous brainstem lesions [34], and a survey of pediatric neurosurgeons conducted by Hankinson et al. found interobserver agreement regarding the classification of a tumor as “typical DIPG” in less than half of the cases [14]. Two of the seven cases corresponded to entities with markedly different management and prognosis (pilocytic astrocytoma and ETMR), which are more likely to fall at the atypical end of our inclusion spectrum and illustrate the broader scope of non-DIPG brainstem tumors in children [18]. Our case 17 illustrates this risk most directly: the patient had been treated empirically at another institution, based on radiological suspicion of DIPG, with chemotherapy for approximately two years, without histological confirmation; due to disease progression, she was referred to us for a biopsy, and the histopathological result confirmed a pilocytic astrocytoma—a tumor with a fundamentally different prognosis, consistent with her stable residual disease and survival at the last follow-up. The remaining five cases were classified as molecularly unconfirmed DIPG because the essential CNS5 criteria for a diagnosis of H3 K27 alteration were not met. Three of these cases (patients 7, 10, and 16) showed loss of nuclear H3K27me3 expression on immunohistochemistry but lacked a confirmatory molecular alteration; according to the 5th edition criteria, the loss of immunohistochemical trimethylation is necessary but not sufficient; a defining molecular event (an H3 mutation, EGFR amplification, EZHIP overexpression, or a compatible methylation profile) is also required. This limitation has two sides. Hotspot sequencing alone may fail to detect non-canonical H3K27 alterations—such as the DIPG/DMG with wild-type H3 and EZHIP overexpression described by Castel et al. [3], which loses H3K27 trimethylation through PRC2 inhibition and exhibits a phenotype similar to that of H3K27M tumors—so that integrated immunohistochemistry is necessary to capture the full spectrum of the disease; on the other hand, isolated immunohistochemical loss cannot, by itself, establish the diagnosis. Although DIPG remains a useful clinical designation, approximately 80% of cases exhibit H3 K27 alterations and are classified as H3 K27-altered diffuse midline glioma [24], while tumors without confirmed alterations likely represent a heterogeneous group of distinct or incompletely characterized entities.

Two of our cases—pilocytic astrocytoma and ETMR—illustrate the clinical relevance of biopsy. The patient with pilocytic astrocytoma underwent a biopsy followed by radiation therapy, without the need for additional surgery, and remains disease-free 30.7 months later. The patient with ETMR received craniospinal irradiation and adjuvant

chemotherapy according to a protocol for embryonal tumors (the Jakacki protocol) and is alive and disease-free 49.6 months after the biopsy—an outcome that would have been inconceivable had the patient been treated as a DIPG case. These two cases, individually, would be considered exceptional in the DIPG literature; both reinforce the principle that radiological diagnosis alone is insufficient for diagnosing pediatric brainstem tumors [14].

The five cases in our cohort of molecularly unconfirmed DIPG may correspond to the subgroups with prolonged survival observed by Mackay et al. [23]. One patient (Patient 19) was enrolled in the CheckMate 908 study, received nivolumab and ipilimumab, and remains alive after 96.7 months, while the others survived between 13.0 and 26.3 months. Two observations may contradict the hypothesis of an unidentified diagnosis of an H3 K27 alteration: the magnitude and duration of the patient's response, which are inconsistent with the universally poor prognosis of DMG H3 K27-altered; and the presence of tumors with a more stable genomic profile (few DNA copy number alterations) or with a profile similar to that of low-grade gliomas with alterations in the MAPK pathway [23, 32]. The targeted panel applied to this patient did not identify variants at key points in the MAPK pathway (negative for BRAF and FGFR1); therefore, the similarity to long-term survivors with MAPK pathway alterations remains hypothetical. In retrospect, this case provides a compelling argument for biopsy beyond the specific molecular issue at hand: the possibility of inclusion in an immunotherapy clinical trial—a possibility that radiographic diagnosis alone may preclude. Recent studies in connectomics have further suggested that the prognosis of DMG may be influenced not only by anatomical location and molecular class, but also by the tumor's connectivity to conserved pediatric brain networks [39]. This emerging picture supports the concept that future patient risk stratification may require combining tissue-based molecular diagnostics with state-of-the-art neuroimaging and network-level biomarkers.

The primary pragmatic rationale for brainstem biopsy in high-income countries is inclusion in biomarker-guided clinical trials, ongoing immunotherapy [41], and CAR T clinical trials [36, 43, 44]. For most Brazilian patients, this argument does not currently apply: to the best of our knowledge, no Brazilian academic center has actively recruited pediatric patients with DIPG for a clinical trial of targeted therapy based on a molecular profile derived from a biopsy, and international trials require recruitment in the patients' countries of origin. Therefore, it is reasonable to question whether a biopsy is justified in

our context.

Our data suggest that biopsy retains clinical utility even in this context on four distinct grounds. First, integrated histopathological and molecular evaluation led to diagnoses other than H3 K27-altered DIPG in more than one-third of our patients, distinguishing among aggressive DIPG with H3 K27 alteration, low-grade glioma, embryonal tumor, and molecularly unconfirmed pontine lesions, each with potentially distinct natural histories. Second, even within the subgroup with H3K27 alterations, the identification of H3.3 versus H3.1 variants and co-occurring molecular alterations—notably ACVR1 mutations frequently observed with HIST1H3B K27M [11]—refines prognostic stratification, providing information of immediate value for family counseling

and palliative care planning. Consistent with this, tumors with the H3.3 (H3F3A) mutation have been associated with clinical and biological characteristics distinct from those of the H3.1 (HIST1H3B/C) subgroup [4], although the prognostic implications should be interpreted with caution, given the small size of our cohort. The prospective study by Puget et al., involving 130 stereotactic DIPG biopsies, also indicated that the WHO histological grade was an unsatisfactory predictor of outcome, whereas the molecular profile provided more clinically meaningful stratification [31]. Third, the systematic storage of fresh-frozen tumor tissue contributes to the institutional infrastructure for the development of preclinical models (patient-derived cell lines and xenografts) under the INOVACRIANCA Project, carried out at our Center for Molecular Oncology Research, supporting translational research on a tumor for which representative preclinical models remains scarce globally [40]. Fourth, the establishment of an integrated workflow within the institution lays the groundwork for the eventual implementation of biomarker-guided clinical trials in Brazil

and contributes archived tissue and molecular data to international consortia. In the long term, a routine biopsy and biobanking program will also build the infrastructure necessary for eventual participation in emerging interventional strategies, including convection-enhanced delivery, MRI-guided focused ultrasound for modulation of the blood-brain barrier, and locoregional approaches using CAR T cells targeting antigens such as GD2 or B7-H3 [19, 36]. We emphasize that none of these modalities are currently available at our center; the point is that the tissue, molecular data, and workflow generated by routine biopsy are prerequisites for future participation in such trials and biobanks. From a broader perspective, these findings suggest that the value of this approach may lie in integrating existing knowledge from microsurgery, standardized tissue handling, molecular pathology, tissue banks, and structured family counseling.

These benefits must be weighed, on a case-by-case basis, against the costs of the procedure: the possibility of a one- to two-week delay in the start of radiation therapy, which is not insignificant in a disease whose clinical course can be rapid; the risk of transient postoperative morbidity, the relative weight of which is magnified by a survival span measured in months rather than years; and the institutional and family burden of an additional surgical episode in an already grueling clinical course. Our results suggest that, in the hands of a team experienced in microsurgery and with access to an integrated molecular workflow, the balance tips in favor of performing the procedure for cases suspected of DIPG; however, this conclusion is contingent upon adequate surgical expertise and a joint decision with the patients' families, made after an explicit discussion of these advantages and disadvantages.

Our experience also provides a useful complement to the only previously published Brazilian series, that of Morais et al. from the University of São Paulo, which reported 26 cases treated with stereotactic surgery between 2008 and 2018, with a diagnostic yield of 84.6% and a similar safety profile [25]. The lower diagnostic rate in the Brazilian stereotactic series—likely related to smaller samples and the absence of routine molecular analyses at that institution at that time—and the higher rate in our microsurgical series suggest that, in the Brazilian context, the combination of a generous tissue sample with an integrated workflow of molecular analyses may be a determining factor in therapeutic management. We do not interpret this as evidence that microsurgical biopsy is superior to stereotaxy; both approaches have their place, and the small, non-randomized samples preclude any such inference. Instead, we emphasize that the value of the biopsy depends as much on how the tissue is handled in subsequent stages as on the surgical technique itself.

Beyond Latin America, our findings are consistent with the broader international experience with brainstem biopsy in resource-limited and non-Western settings. Lim et al. reported a series of 21 patients with intrinsic brainstem tumors who underwent biopsy at Singapore Children's Hospital between 2006 and 2021. The authors used a mixed approach (10 open procedures and 11 stereotactic procedures); they observed no negative diagnostic results, no permanent postoperative complications, or perioperative mortality, and next-generation sequencing for treatable mutations was subsequently performed in five cases [21]. This Asian series used biopsy both to establish a histological diagnosis and to enable molecular analysis aimed at targeted therapy, supporting the view that the diagnostic and translational value of brainstem biopsy is reproducible across various healthcare systems when associated with an integrated workflow between pathology and molecular analysis.

The decision to perform a biopsy has implications that go beyond the immediate clinical issue. In a qualitative study of 24 families of children who died from DIPG, De Clercq et al. found that most parents did not regret the decisions they made (whether in favor of or against treatment) when those decisions were based on information and aligned with their stated values; on the other hand, families who felt passive or insufficiently informed were more likely to experience complicated grief [9]. Although the parents' experience was not assessed in this cohort, the qualitative literature suggests that structured, values-aligned decision-making can reduce subsequent regret. In this context,

biopsy should be viewed neither as mandatory nor as futile, but as a preference-sensitive option, explicitly discussed in terms of its expected diagnostic value, procedural risk, and subsequent utility.

Limitations

We acknowledge several limitations in our study. This is a retrospective, single-center series with a modest sample size, presenting the inherent risks of selection bias and limited statistical power: the confidence intervals around the Kaplan-Meier estimate are wide, and the small size of the subgroups precludes a formal comparison of survival; therefore, differences between subgroups are presented descriptively (swimmer plot) and should be considered exploratory. The molecular panel was not uniform throughout the study period, evolving from Sanger sequencing of hotspots to NGS-based panels with progressively broader coverage; this likely explains, at least in part, the observed diagnostic heterogeneity. Immunohistochemistry for H3K27me3 and EZHIP, EGFR mutation testing, and whole-genome DNA methylation profiling were not performed systematically; therefore, the H3K27 mutation could not be definitively ruled out in the two molecularly unconfirmed cases. The cohort also included newly diagnosed patients alongside those previously treated at other centers (patients 13 and 17), which introduced heterogeneity; a sensitivity analysis excluding the previously treated patients was not performed due to the small sample size. The data were not centrally reviewed by a blinded neuroradiologist. The surgical approach used in this series relies on specific knowledge and experience in microneurosurgery, limiting the direct generalizability of the safety data to other centers. The small, non-randomized design does not allow for any inference of an advantage over stereotactic biopsy. Length of hospital stay was retrospectively extracted from hospital records and may reflect institutional discharge practices and the geographic dispersion of our referral base, rather than solely the clinical recovery profile following the procedure; comparisons between series should take into account the heterogeneous, often unreported criteria used to define discharge. Finally, the present cohort was not designed to study the psychosocial impact of the procedure on patients or their families; the discussion of these dimensions is based on published qualitative literature, rather than on data from our own series.

Conclusion

In this article, we report our experience performing biopsies on 19 pediatric patients with radiologically suspected DIPG using open microsurgery. We were able to obtain tumor tissue in 100% of cases, with no permanent morbidity and no mortality associated with the procedure. We found a final diagnosis other than DMG H3 K27-altered molecularly confirmed, including one child with pilocytic astrocytoma and another with an embryonal tumor with multilayered rosettes who remain alive at the last follow-up, and one with a molecularly unconfirmed DIPG who has survived for more than eight years on immunotherapy. Even though we did not have locally available clinical trials for targeted therapy, the integrated histopathological and molecular evaluation of brainstem tumors altered clinical management or enabled experimental therapy in a clinically significant proportion of our cases. These data can serve as a basis for the selective implementation of brainstem biopsy in referral centers in Latin America, provided they are accompanied by adequate microsurgical expertise and access to an up-to-date molecular workflow, and they illustrate a path toward implementation in global neurosurgery based on existing microsurgical knowledge.

Declarations

Ethics approval and consent to participate. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethics Committee of Barretos Cancer Hospital and with the

1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. This retrospective single-center study was approved by the Institutional Ethics Committee of Barretos Cancer Hospital (Protocol number 78910824.6.1001.5437).

Research involving animals. This study did not involve experiments on animals.

Informed consent. Informed consent was obtained from the legal guardians of all participants included in the study.

Consent for publication: Not applicable

Availability of data and material. The data supporting the findings of this study are available from the corresponding author upon reasonable request. All data requests will be considered and must be reviewed and approved by the relevant ethics committee. The custom Python script used to compute the survival estimates and generate the figures is also available from the corresponding author upon reasonable request.

Conflict of interest. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Figures

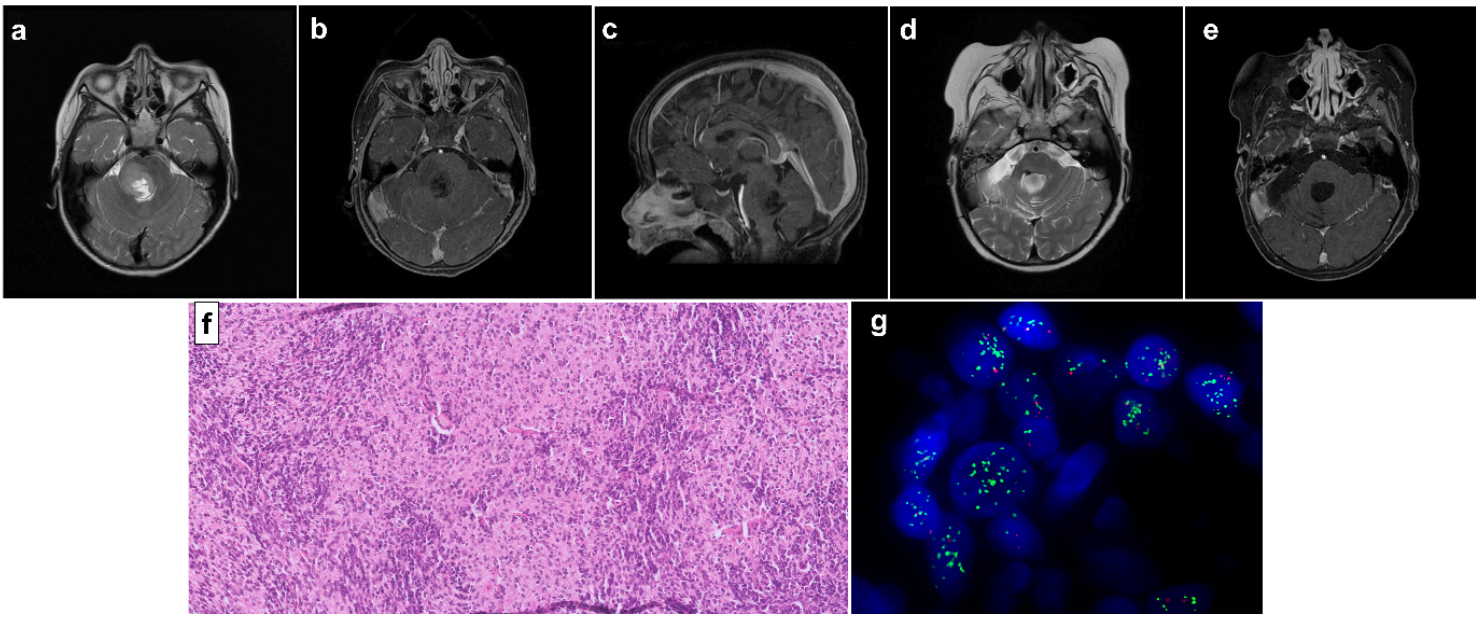


Figure 1

Magnetic resonance Imaging, histology and FISH of a patient with radiologically suspected DIPG. a-c At presentation (April 2022). **a** Axial T2-weighted image. **b** Axial T1-weighted image. **c** Sagittal T1-weighted image after gadolinium injection. **d-e** At last follow-up (May 2026). **d** axial T2-weighted image. **e** axial T1-weighted image after gadolinium injection. **f**ETMR_HE: Histological examination reveals a small round cell neoplasm composed of clustered tumor cells admixed with neuropil-rich areas and exhibiting neurocytic differentiation (20X objective). **g** Fluorescence in situ hybridization (FISH) showing amplification of the C19MC locus at 19q13.42 (green signal) in tumor cell nuclei relative

to the reference probe at 19p13.3 (orange signal). Nuclei are counterstained with DAPI (blue). Magnification: 100× objective (oil immersion). Histological diagnosis embryonal tumor with multilayered rosettes (ETMR), C19MC-amplified.

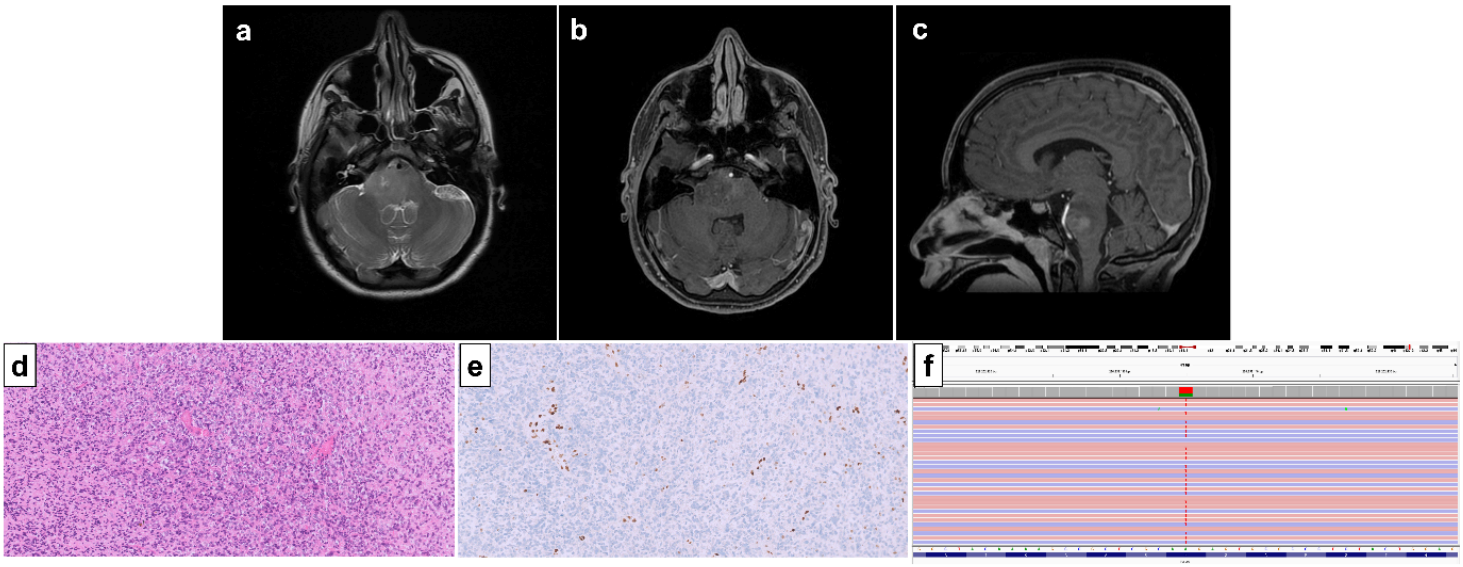


Figure 2

Magnetic resonance Imaging, histology, IHC (H3K27me3) and NGS (H3F3A) of a patient with radiologically suspected DIPG. **a** Axial T2-weighted image. **b** Axial T2-FLAIR image. **c** Sagittal T1-weighted image after gadolinium injection. Histological diagnosis diffuse midline glioma, H3 K27-altered. **d**H3F3A_HE: Histological examination reveals a glial neoplasm of high cellularity exhibiting an oligodendroglioma-like morphology, characterized by perinuclear halos (20X objective). **e** H3K27me3_H3F3A: Immunohistochemical examination demonstrates loss of H3K27me3 expression in tumor cells, with retained expression in endothelial cells (20X objective). **f** Integrative Genomics Viewer (IGV) visualization of next-generation sequencing (NGS) data showing the H3F3A (H3.3A) c.83A>T, p.(Lys28Met) (NM_002107.7) variant, commonly referred to as K27M. The figure illustrates the pathogenic amino acid substitution of lysine (K) by methionine (M) at codon 28 of histone, resulting from an adenine-to-thymine nucleotide change at position 83.

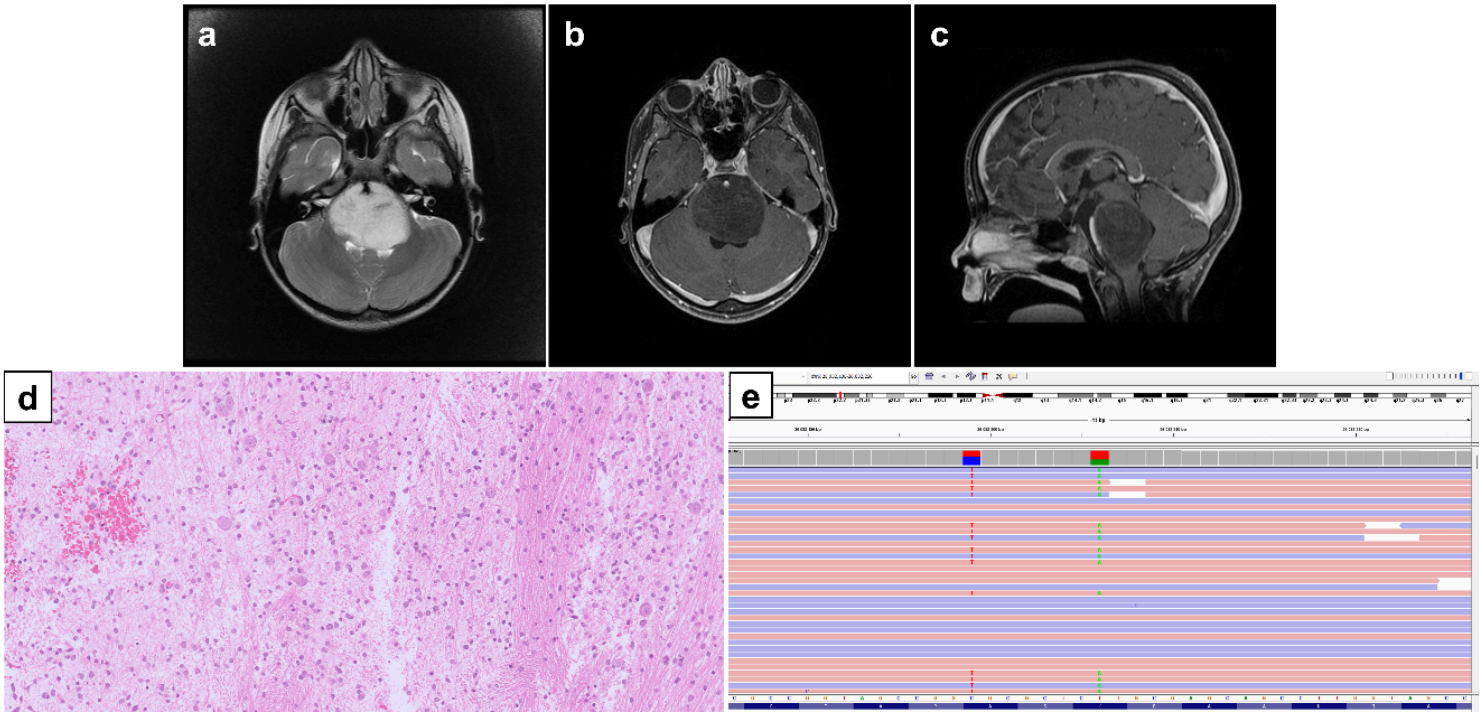


Figure 3

Magnetic resonance imaging, histology, NGS (HIST1H3B) of a patient with radiologically suspected DIPG. **a**Axial T2-weighted image. **b** Axial T1-weighted image. **c** Sagittal T1-weighted image after gadolinium injection. Histological diagnosis diffuse midline glioma, H3 K27-altered. **d** HIST1H3B_HE: Histological examination reveals an infiltrative glial neoplasm with astrocytic morphology, characterized by diffuse growth of tumor cells among native neurons (20X objective). **e** Integrative Genomics Viewer (IGV) visualization of next-generation sequencing (NGS) data showing the HIST1H3B (H3C2) c.83A>T, p.(Lys28Met) (NM_003537.3) variant, commonly referred to as K27M. The figure illustrates the pathogenic amino acid substitution of lysine (K) by methionine (M) at codon 28 of histone, resulting from an adenine-to-thymine nucleotide change at position 83.

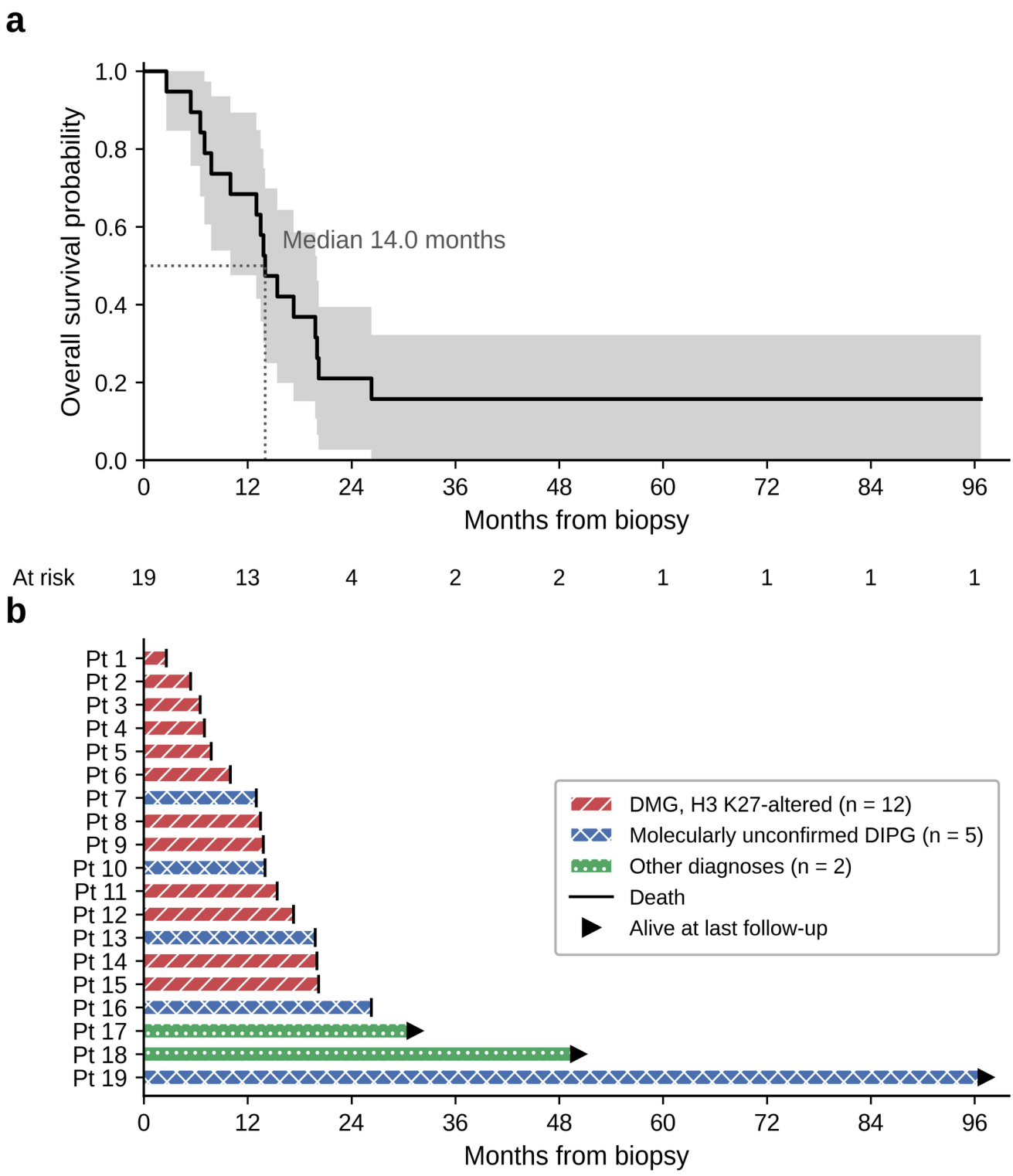


Figure 4

Overall survival after microsurgical brainstem biopsy. **a Kaplan-Meier** estimate of overall survival in the entire cohort (n = 19); the shaded area represents the 95% confidence interval and the dotted lines indicate the median overall survival of 14.0 months; numbers at risk are shown beneath the panel. **b Swimmer plot** of individual overall survival by histomolecular subgroup: diffuse midline glioma, H3 K27-altered (n = 12), molecularly unconfirmed DIPG (n = 5), and other diagnoses (pilocytic astrocytoma and embryonal tumor with multilayered rosettes; n = 2); each bar represents one patient, vertical caps denote death, and arrowheads denote patients alive at last follow-up

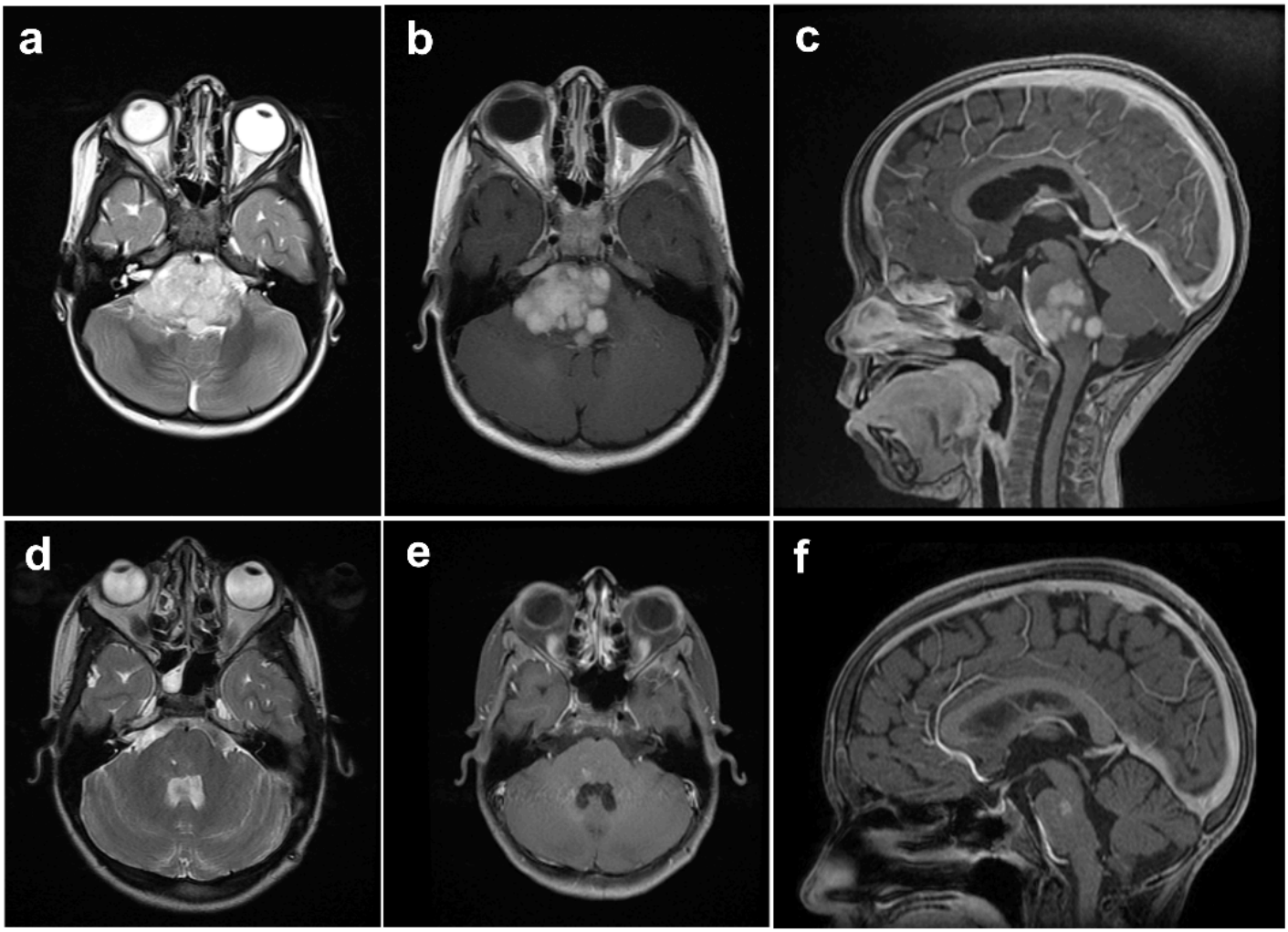


Figure 5

Magnetic resonance imaging of Patient 19 (molecularly unconfirmed DIPG). **a-c**: At presentation (April 2018). **a** axial T2-weighted image; **b** axial T1-weighted image; **c** sagittal T1-weighted image after gadolinium injection. **d-f**: At last follow-up (May 2026). **d** axial T2-weighted image. **e** axial T1-weighted image. **f** Sagittal T1-weighted image after gadolinium injection. Following 54 Gy of focal radiotherapy, the patient was enrolled in the CheckMate 908 trial and treated with nivolumab plus ipilimumab, and remains alive with stable residual disease 96.7 months after biopsy.