Original Paper

# Pathological perspectives in pilocytic astrocytomas: Extent of resection as the sole critical factor for recurrence-free survival, and the challenge of evaluating conclusions derived from limited data

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Submitted: 17 September 2023 · Accepted: 09 October 2023 · Copyedited by: Lauren Walker · Published: 20 October 2023

## Abstract

**Introduction:** Pilocytic astrocytoma (PA) is one of the most common primary intracranial neoplasms in childhood with an overall favorable prognosis. Despite decades of experience, there are still diagnostic and treatment challenges and unresolved issues regarding risk factors associated with recurrence, most often due to conclusions of publications with limited data. We analyzed 499 patients with PA diagnosed in a single institution over 30 years in order to provide answers to some of the unresolved issues.
**Materials and Methods:** We identified pilocytic astrocytomas diagnosed at the University of California, San Francisco, between 1989 and 2019, confirmed the diagnoses using the WHO 2021 essential and desirable criteria, and performed a retrospective review of the demographic and clinical features of the patients and the radiological, pathologic and molecular features of the tumors.
**Results:** Among the patients identified from pathology archives, 499 cases fulfilled the inclusion criteria. Median age at presentation was 12 years (range 3.5 months – 73 years) and the median follow-up was 78.5 months. Tumors were predominantly located in the posterior fossa (52.6%). There were six deaths, but there were confounding factors that prevented a clear association of death to tumor progression. Extent of resection was the only significant factor for recurrence-free survival. Recurrence-free survival time was 321.0 months for gross total resection, compared to 160.9 months for subtotal resection (log rank, p <0.001).

**Conclusion:** Multivariate analysis was able to identify extent of resection as the only significant variable to influence recurrence-free survival. We did not find a statistically significant association between age, *NF1* status, tumor location, molecular alterations, and outcome. Smaller series with apparently significant results may have suffered from limited sample size, limited variables, acceptance of univariate analysis findings as well as a larger p value for biological significance. PA still remains a predominantly surgical disease and every attempt should be made to achieve gross total resection since this appears to be the most reliable predictor of recurrence-free survival.

**Keywords:** Astrocytoma, Entity, Circumscribed gliomas, Glioma, Juvenile pilocytic astrocytoma, Pilocytic astrocytoma, Piloid, Pilomyxoid, Tumor type

## Introduction

Pilocytic astrocytoma (PA) is a circumscribed astrocytoma with classic histologic features such as biphasic compact and loose growth patterns, piloid cytology, and low proliferative activity, with or without Rosenthal fibers and/or eosinophilic granular bodies [1]. The essential criteria adopted by the World Health Organization (WHO) 2021 also defines PA as a "piloid astrocytic neoplasm with a solitary MAPK pathway alteration, such as *KIAA1549::BRAF* fusion" [2]. Over the last century, beginning with the first use of the word "pilocytic" [3] WHO classification schemes defined PA as a clinically, radiologically, pathologically, and most recently, a molecularly distinct entity. Typical radiological and histological features are presented in Figure 1.

PA can be observed at any age with a reported incidence rate of approximately 0.84 per 100,000 [4-6], and has a favorable outcome [5]. Despite being the most common glioma among the pediatric population, overall rarity of this neoplasm makes it an orphan disease as well as a "chronic disease" affecting patients and families for many years, creating challenges in its management [7-11].

Coding of PAs in tumor registries has been problematic in recent years due to their designation as "malignant" in some countries. This change was made in order to capture these tumors in cancer registries [12]. Consequently, incidence of benign or malignant pediatric brain tumors show significant variations over time in epidemiological analyses [13]. We are not sure whether designating PAs as "malignant" in order to capture them in registries is a good idea, or whether this practice should be abandoned to bring more clarity and uniformity to the issue worldwide, but risk losing their identification in cancer statistics.

PA is most commonly located in the posterior fossa, specifically in cerebellar hemispheres. Other common locations include hypothalamic/chiasmatic region and the optic nerve, the latter being particularly common in the setting of Neurofibromatosis 1 syndrome [14]. Thalamic, cerebral, and spinal tumors are distinctly less common [5]. Some authors suggested that there were prognostic differences among PAs in different locations that could not be explained simply by differences in clinical variables [15-17]. It is not clear whether different locations are associated with different outcomes beyond access to gross total resection, and whether tumor location is associated with different driver mutations and treatment response.

In addition to location, some studies suggest that age is important determinant of outcome, and adult and pediatric PAs do not have similar prognoses [18-21].

PAs can have diverse morphological features, some of which may result in misclassification of some tumors as diffuse gliomas. Some PAs with the so-called "diffuse pilocytic pattern" may be easily misdiagnosed as diffuse gliomas [22]. Conversely, other entities may also be misclassified as PA and some high-grade tumors with remote resemblance to PA may be erroneously classified as tumors with piloid features or simply anaplastic PA [23]. High-grade histological features in otherwise typical PAs are also problematic and the current WHO classification scheme did not find sufficient evidence to define an anaplastic subtype [24]. However, there is

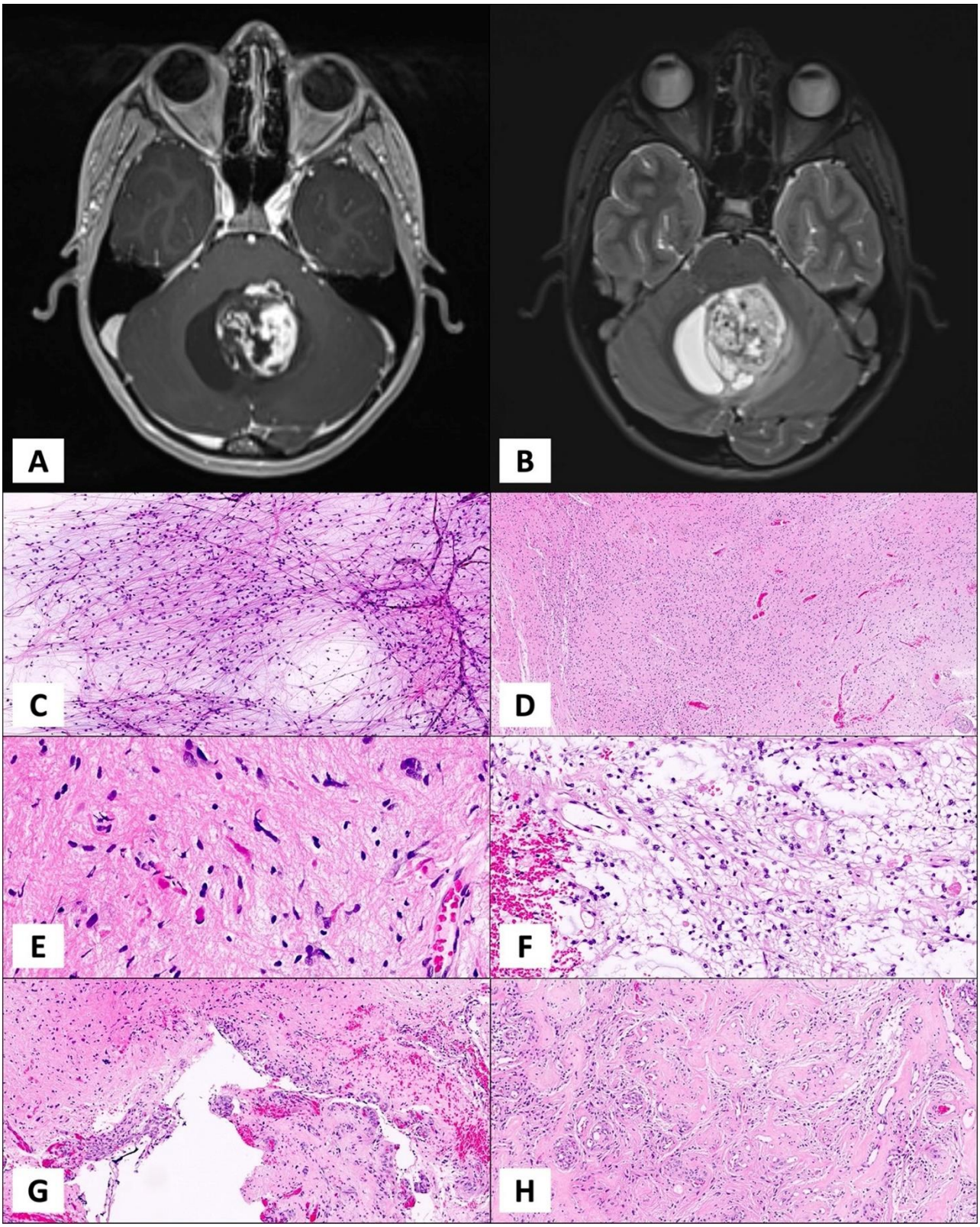


**Figure 1.** A cystic tumor with mural nodule in posterior fossa on axial T1 post-contrast (A) and T2 (B) weighted MRI images. An intraoperative smear slide of a PA case with plenty of piloid cells distributed on a glial background (C). Hematoxylin & eosin image of a demonstrative PA case with an overall fibrillary appearance (D), Closer view with plenty of Rosenthal fibers, classical finding in PAs (E). Myxoid, loose areas are another common finding in PA (F). Linear glomeruloid vascular structures are commonly observed (G) along with hyalinized, thick vessels (H).

clear evidence that rare PAs behave aggressively and some of these tumors may be justifiably designated as high-grade gliomas [25]. On the other hand, some worrisome histological features in PA have not been associated with adverse prognosis [26-28]. Additional studies and observations are needed to validate a high-grade subtype of PA and its definition.

Currently, the only recognized subtype of PA is the pilomyxoid astrocytoma (PMA), that appears to be more aggressive compared to typical PAs [29]. However, in the recent editions of the WHO classification of central nervous system (CNS) tumors, the working groups decided that the data for recognizing this subtype as more aggressive were not sufficient to designate a specific WHO grade for PMA [15, 29, 30]. Further studies are needed for the pilomyxoid tumors to allow their grading and prognostication.

Recent genomic studies outline the molecular landscape of PAs as tumors with a very stable genome, carrying less than 0.1 mutations per megabase [31, 32]. Mitogen activated protein kinase (MAPK) pathway is the most commonly affected pathway in PAs. The most common genetic alteration is the *BRAF* internal tandem duplication resulting with a fusion of a neighboring gene, *KIAA1549*. Other alterations of the MAPK pathway, such as other *BRAF* fusions, mutations in *BRAF, RAS* or *NF1* were also reported [2, 33-39]. While the type of MAPK alterations has not been associated with different outcomes, it is not clear whether the cumulative effect of multiple genetic alterations may lead to a more aggressive behavior in PAs. Some studies implicated molecular alterations such as loss of *CDKN2A*, gain of chromosome 7 and loss of chromosome 17q as being associated with worse outcome, but none of these studies were validated in large series, prospective trials or by more than one group [25, 40].

Several clinical and biological markers reportedly influence prognosis in patients with PA, but these parameters, with the exception of extent of resection, have not been consistently found as independent variables [26-28, 41]. We also suggested that access to healthcare is a key factor in the outcome of patients with PA, most likely unrelated to tumor biology [42].

In this retrospective study, we report our experience with 499 PAs diagnosed and treated in a single institution over the last three decades in order to bring clarity to some of the uncertainties mentioned above, and to further demonstrate the need for collaborative studies and larger series to better understand factors associated with outcome.

## Material and Methods

### Patient selection

All patients diagnosed as PA or PMA between 1989 and 2019, were retrieved from the pathology archives of the Department of Pathology. The inclusion criteria were as follows: 1- all patients diagnosed and/or treated at our institution between 1989 and 2019, and 2- sufficient clinical information to include into the database, and 3- sufficient pathology material available for review and diagnosis, and 4- the diagnosis of PA or PMA upon review of the available material. The database search included a series of keywords for final diagnoses, and the search was conducted at two separate occasions, which yielded nearly identical results with a rare exception due to delay in the database registry. Clinical and radiological information were obtained from the hospital electronic information systems or from the patient charts for older cases. All relevant clinical information necessary for the purposes of our analyses were collected in an anonymized fashion and a research database was created. Radiological reports were also reviewed to ensure that radiological impression was consistent with PA. Extent of resection was obtained from the operative reports and was reported as either gross total resection (GTR) or subtotal resection (STR). Any level of resection less than GTR was considered STR. Recurrence was defined as worsening of clinical symptoms attributed to tumor growth. Recurrence-free survival (RFS) was calculated between the date of the initial surgery and the first record of clinical worsening in the chart. Overall survival was calculated as the time between the initial surgery and death due to tumor, and all others were censored. The cut-off for the follow-up time was January 2022. Patients who could not be identified in the system by that date were considered lost

to follow-up. This study was approved by the University of California San Francisco Institutional Review Board (IRB approval no. CHR 10-01252).

### Pathological evaluation

All available pathology material from all cases were reviewed by two of the authors (IK, TT) and one of the authors (MP) contributed to the pathological review process for the recent cases during the last decade. PA and PMA diagnoses were based on the essential and desirable criteria proposed by the WHO 2021 classification scheme. Pertinent histopathological features were recorded. Immunohistochemical studies were performed as a part of the routine work-up of cases to establish diagnosis and further characterize the histological features.

### BRAF V600 mutation analysis by real time PCR

A total of 222 patient samples were submitted to BRAF V600E mutational analysis by real time PCR as a part of their routine diagnosis. Real time PCR was performed according to published methodology at the Clinical Cancer Genomics Laboratory that operates under a CLIA license. Appropriate controls and quality assurance parameters have been established at this laboratory (https://genomics.ucsf.edu/content/braf-mutation-testing-including-v600e).

### BRAF duplication / KIAA1549::BRAF fusion analysis

In limited number of cases, BRAF duplication only was investigated via fluorescence in-situ hybridization (FISH) as previously described as a part of the patients' routine diagnostic work-up at the Clinical Cancer Genomics Laboratory (https://genomics.ucsf.edu/content/braf-rearrangement-fish).

### Analysis of molecular alterations and copy number variations (CNV)

Capture-based next-generation DNA sequencing (NGS) was performed at the UCSF Clinical Cancer Genomics Laboratory (also referred as UCSF500 NGS assay) in 106 patients as a part of their routine diagnostic work-up according to protocols described previously [43]. Further information on the specifics of the UCSF500 NGS platform is available at the CCGL website (https://genomics.ucsf.edu/content/ucsf-500-cancer-gene-panel-test-ucsf500-uc500).

### Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows (IBM Corp., Version 28.0, released 2021, Armonk, NY: IBM Corp). The normality of continuous variables was investigated by Shapiro-Wilk's test. Descriptive statistics were presented using mean, standard deviation, median and interquartile range. Categorical variables were expressed by using frequencies (n) and percentages (%). To compare categorical variables Chi-Square test (or Fisher exact test/Yates continuity correction as appropriate) was used. The recurrence-free survival was evaluated by Kaplan-Meier method and the median survival times were compared by log rank test. The associations between clinicopathologic features and the recurrence free survival were evaluated by Cox regression model. The cut off for statistical significance was set as $p<0.001$.

## Results

### Demographic and clinical features

Among a total of 528 patients identified from the database with the diagnosis of PA or PMA between 1989-2019, 499 patients fulfilled the inclusion criteria and were included in this study. Mean age at the diagnosis was 15.5 years (range: 3.4 months-72 years) and the median age was 12 years. There were 276 females (55.3%) and 223 males (44.7%). The patients were grouped into three age categories; 286 patients were $\leq$14 years old, 77 patients were between 14-21 years old, and 136 cases were >21 years old.

The median follow-up was 78.5 months (1 – 580 months) and 47 (9.4%) patients had a follow-up period less than 12 months. In addition, 178 patients had $\geq$5 years and 114 patients had $\geq$10 years of follow-up.

Non-surgical treatment data were available on approximately half (n=298) of the cases and among them, 82 patients have received chemotherapy and 63 have received radiotherapy.

**Table 1. Clinical details of deceased patients**

| ID | Patient Age at Sx | Location | Treatment | Comorbidities | Molecular Findings | OS |
|---|---|---|---|---|---|---|
| Case# 29 | 5 years | Cerebral | 4 surgeries<br>WBRT<br>Multiple chemotherapies | Hemiparesis. Seizures. Panhypopituitarism Radiation-associated necrosis*. Stable tumor in last neuroimaging study | N/A | 24 yr |
| Case# 49 | 1.3 years | Hypothalamic/ Chiasmatic | 2 surgeries<br>Multiple chemotherapies<br>External beam radiation<br>11 surgical procedures | Radiation-associated necrosis*. Seizures. Hydrocephalus. Secondary infection-sepsis. | N/A | 11 mo |
| Case# 86 | 49 years | Posterior Fossa | 2 surgeries<br>External beam radiation<br>Multiple chemotherapies | Radiation-associated necrosis*. Hemiparesis Hydrocephalus. SIADH. Stable tumor in last neuroimaging study | N/A | 2 yr |
| Case #177 | 39 years | Spinal Cord | 2 surgeries<br>Radiation treatment<br>Multiple chemotherapies | Radiation associated necrosis*. Paraplegia Neurogenic bladder, recurrent UTI. Papillary urothelial carcinoma. Stable tumor in last neuroimaging study. | N/A | 14 yr |
| Case# 249 | 4 years | Hypothalamic/ Chiasmatic | 2 surgeries<br>Radiation Treatment<br>Multiple chemotherapies<br>Multiple VP shunting | Hydrocephalus. Radiation-associated necrosis*. Seizures. Cerebral infarction. SIADH. Dysphagia and aspiration pneumonia | *BRAF::KIAA1549* | 11 yr |
| Case #454 | 35 years | Posterior fossa | 3 surgeries<br>Radiation treatments (3)<br>Multiple chemotherapies<br>Multiple shunt revisions | Neurofibromatosis 1. Aspiration pneumonia Multiple neurofibromas. Radiation-associated necrosis*, cerebellum. Multiple FLAIR abnormalities in the cerebral hemispheres.<br>No tumor growth reported following third surgery on radioimaging | *NF1* loss somatic /germline CDKN2A homozygous deletion in recurrent tumor | 6 yr |

- Radiation-associated necrosis was confirmed on radioimaging as well as pathological studies of the recurrent tumors.
- WBRT- whole brain radiation; VP: ventriculoperitoneal; SIADH: syndrome of inappropriate ADH secretion; UTI: urinary tract infection; OS: overall survival.

Six patients died during the follow-up period, but detailed analysis of their disease course could not directly establish cause of death as being due to tumor. All six patients initially underwent subtotal resections, received radiotherapy and multiple chemotherapy regimens, and had significant comorbidities (see Table 1). In most cases, there was no clear tumor growth, and the disease course was complicated by additional factors.

Tumor recurrence information was available for 321 patients, of which 109 suffered at least one recurrence (34%, 109 of 321 cases). Major demographic and clinical features are presented in table 2.

## Tumor localization

Among the cases where the localization information was available (n=494), 259 (52.6%) were in the posterior fossa, 208 (42.3%) were supratentorial (includes hemispheric, hypothalamic/chiasmatic, intraventricular and optic nerve tumors), and 25 (5.1%) were in the spinal cord. Two patients had multifocal tumors at diagnosis, and the exact information on location was not available for 5 patients.

Among 208 supratentorial tumors, 108 (51.9%) were in the cerebral hemispheres, 79 (38%) were in the hypothalamic/chiasmatic region, 6 (2.9%) were intraventricular, and 15 (7.2%) were involving the optic nerve. While there was a suggestion that

**Table 2. Major demographic and clinical features of patients**

| VARIABLE | GROUPS | N | % |
|---|---|---|---|
| AGE n:499 | 0-14 | 286 | 57.3% |
| | 14-21 | 77 | 15.4% |
| | >21 | 136 | 27.3% |
| SEX n:499 | Male | 276 | 55.3% |
| | Female | 223 | 44.7% |
| EXTENT OF RESECTION n:499 | STR | 235 | 47.1% |
| | GTR | 169 | 33.9% |
| | Unknown | 95 | 19.0% |
| TUMOR LOCALIZATION n:499 | Infratentorial | 259 | 51.9% |
| | Supratentorial | 208 | 41.6% |
| | Spinal | 25 | 05.0% |
| | Multifocal | 2 | 0.5% |
| | Unknown | 5 | 01.0% |
| TREATMENT n:499 | Chemotherapy | | |
| | *Yes* | 82 | 16.4% |
| | *No* | 216 | 43.3% |
| | *Unknown* | 201 | 40.3% |
| | Radiotherapy | | |
| | *Yes* | 63 | 12.6% |
| | *No* | 235 | 47.1% |
| | *Unknown* | 201 | 40.3% |
| OUTCOME n:499 | No Evidence of Disease | 80 | 16.0% |
| | Alive with Disease | 62 | 12.4% |
| | Lost, to Follow Up | 351 | 70.3% |
| | Died of Other Causes | 6 | 01.2% |
| RECURRENCE n:499 | Yes | 109 | 21.8% |
| | No | 212 | 42.5% |
| | Unknown | 178 | 35.7% |
| SECOND RECURRENCE n:109 | Yes | 20 | 18.3% |
| | No | 58 | 53.2% |
| | Unknown | 31 | 28.5% |

n: Total number of patients; STR: subtotal resection; GTR: gross total resection.
Second recurrence is reported for 109 patients who had documented recurrences.

posterior fossa tumors were less likely to recur compared with hypothalamic/chiasmatic tumors, there was no statistical significance (p=0.043) partly due to the small number of cases for each location category when controlled for other variables. Even after regrouping cases into “hypothalamo-suprachiasmatic+brainstem" and "other locations", we did not find statistically significant correlation with recurrence rates (p=0.004), or with RFS. Location was not found to be significant on multivariate analysis, suggesting that it is a dependent variable.

Hypothalamic/midline tumors are difficult to reach and harder to remove completely or there may be additional confounding factors and our dataset may not be large enough to tease out these specific factors, so that a significance may be observed in larger studies with longer follow-up. There was no statistically significant difference in RFS between infratentorial and supratentorial tumors, with supratentorial tumors having a minimally higher recurrence rate on univariate analysis that disappeared on multivariate analysis.

## Histological and molecular/genetic features

We identified 6 patients with PMA, and 6 other cases with histological evidence of anaplasia on pathological evaluation. The remaining 487 demonstrated histological features diagnostic of PA, occasionally aided by immunohistochemical evaluation. It was not possible to make a statistical analysis due to limited numbers of cases with unusual histological features. Likewise, a meaningful analysis of RFS for PMA and tumors with anaplastic histology could not be made. Salient features of PMAs and tumors with anaplastic histology are presented on tables 3 and 4, respectively. Histological features were critical for the recognition of PA, but their analysis did not reveal statistically meaningful associations (data not shown).

Because of the retrospective nature of this study and due to the long period of study, the type of molecular testing significantly varied within the group. Tumors from earlier years were tested either with Sanger sequencing for *BRAF* mutations or with FISH for *BRAF* duplication (n=191). In addition, 106 more recent cases were analyzed with a UCSF 500 NGS platform. Twenty-four of the cases had confirmed germline *NF1* mutation (while *NF1* mutations were detected in 5 additional tumors, the UCSF500 NGS platform was conducted solely on tumor tissue in these cases, making it impossible to determine

**Table 3. Clinical and pathological features of patients with tumors showing anaplastic histology**

| | Age/Sex | Localization | Surgery Type | Diagnosis | Mutational Profile | Initial Pathology/ History | Chr Loss | Chr Gains | Recurrence/ Time To Rec (months) |
|---|---|---|---|---|---|---|---|---|---|
| **Case# 226** | 42/F | Hemispheric | Gross total resection | Pilocytic astrocytoma with anaplastic features | *BRAF::KIAA1549* Fusion, CDKN2A Loss, ATRX and NF1 mutations | History of PA, grade 1 (12 years ago) | 2q, 8p, 9q, 10p, 11p, 14q | 2p, 3q, 7q | Yes / 146 |
| **Case# 384** | 46/M | Posterior Fossa | Subtotal resection | Pilocytic astrocytoma with anaplastic features | *FGFR1, PTPN11* mutations, *CDKN2A/B* loss, *ATRX* mutation, *PIK3R1* mutation | History of a posterior fossa tumor at childhood with unknown histology | 2q, 8p, 9p, 10q, 13q, 22q | 8q | Yes / 6 |
| **Case# 454** | 35/F | Posterior Fossa | Subtotal resection | Pilocytic astrocytoma with anaplastic features | *NF1* and *CHEK2* mutations, *CDKN2A/B* loss (in recurrent tumor) | History of PA, grade 1 (4 years ago) | 1, 4p, 5q, 9p, 10q, 13q, 16p | 2p, 4p, 12q, 22q | Yes / 35 |
| **Case# 362** | 27/F | Posterior Fossa | Subtotal resection | Pilocytic astrocytoma with anaplastic features | Germline *NF1* mutation, *ATRX* mutation,*CDKN2A/B* loss | History of PA, grade 1 (22 years ago) | None | None | Lost FU / No information |
| **Case# 488** | 54/M | Posterior Fossa | Subtotal resection | Pilocytic astrocytoma with anaplastic features | *CDK4 and GAB2* amplification, *TP53* mutation | History of a posterior fossa tumor with unknown histology | 5q, 10q, 18p, 20p | 4, 8p, 8q, 9q, 12p, 16, 17q, 18q, 21q | Lost FU / No information |
| **Case# 347** | 73/M | Hemispheric | Subtotal resection | Pilocytic astrocytoma with anaplastic features | N/A | Concurrent PA morphology. History of unknown brain tumor. | | | Lost FU / No information |

whether these mutations were germline or somatic.) One of the tumors harbored both *FGFR1* and *NF1* mutations. The distribution of mutations is displayed in table 5.

Among 199 cases that had molecular analysis performed, the majority (n=150, 75.4%) had alterations in *BRAF* gene (either p.V600 mutations, internal tandem duplication and or *KIAA1549::BRAF* fusion); and the majority of the rest had alterations in other components of MAPK pathway. Because of the small number of tumors in other groups, it was not possible to make a statistical analysis between the type of mutation and RFS.

We analyzed whether tumors with distinct genetic alterations cluster in particular locations. As anticipated, *BRAF* alterations were the most frequent type of mutations in all locations. However, BRAF alterations were more frequent in posterior fossa tumors (81%) in comparison to supratentorial tumors (69.4%; p<0.001). Notably, *FGFR1* mutations were primarily observed in supratentorial tumors, with seven out of eight cases located in the hemispheric, hypothalamic-suprachiasmatic, or intraventricular regions. Although the number of cases was limited, all seven cases with mutations in other components of the MAPK pathway (*KRAS*, *RAF1, SOS1*) were supratentorial. Table 6 shows the distribution of mutations across tumor locations.

Homozygous loss of *CDKN2A* has been reported as one of the poor prognostic factors in the literature [44]. Among 106 patients where this information was available, *CDKN2A* homozygous loss occurred in only 4 patients. Histologically, all four tumors had anaplastic histologic features, typical

**Table 4. Clinical and pathological features of patients with pilomyxoid astrocytoma**

| | Age/Sex | Localization | Surgery Type | Mutational Profile | Chr Loss | Chr Gains | Recurrence/ Time To Rec (months) |
|---|---|---|---|---|---|---|---|
| **Case# 237** | 1.5/M | Hypothalamic-Suprachiasmatic | Subtotal resection | *BRAF::KIAA1549* Fusion | None | None | Yes / 77 |
| **Case# 315** | <1/F | Hypothalamic-Suprachiasmatic | No information | *BRAF::KIAA1549* Fusion | None | None | No |
| **Case# 345** | 6/F | Hypothalamic-Suprachiasmatic | Subtotal resection | *KRAS* p.Q61L | None | None | Lost FU / No information |
| **Case# 366** | 9.1/F | Posterior Fossa | Gross total resection | *BRAF::KIAA1549* Fusion | None | 11 | Lost FU / No information |
| **Case# 379** | 8.3/F | Multifocal | Subtotal resection | *BRAF* p.599dup | None | 5, 6, 7, 8, 11, 12, 14, 16, 18, 20 | Yes / 1 |
| **Case# 382** | 8.5/F | Hypothalamic-Suprachiasmatic | Subtotal resection | *FGFR1* and *PTPN11* mutations | None | 8, 12 | No |

**Table 5. Mutations in 199 pilocytic astrocytomas**

| Mutation Type | N (%) |
|---|---|
| *BRAF* Fusion/Duplication | 114 (57.3%) |
| *BRAF* V600 Mutations | 36 (18.1%) |
| *NF1* Alterations (germline + somatic) | 29 (14.6%) |
| FGFR1 Alterations | 8 (4%) |
| *RAF1* Alterations | 3 (1.5%) |
| *KRAS* Alterations | 2 (1%) |
| *SOS1* Mutation | 1 (%0.5) |
| CDK4 & *GAB2* Amplification | 1 (%0.5) |
| *FGFR1* + *NF1* Alterations | 1 (%0.5) |
| No alterations identified | 4 (2%) |
| Total | 199 (100%) |

MAPK driver mutations (*NF1* mutation in two cases; *FGFR1* mutation in, one case; *KIAA1549::BRAF* fusion in one case), additional molecular changes (*ATRX* mutations in three cases) and multiple chromosomal gains and losses (see Table 3). All four patients had suffered recurrences with a median time to recurrence of 35.5 months (6, 35, 35, 146 months). In 102 tumors without *CDKN2A* alterations (102/106), 26 of the tumors recurred with a median RFS of 32 months (1 - 269 months).

Chromosomal copy number variation (CNV) analysis was done in 106 cases and showed at least one CNV in 39 tumors (36.8%). Nineteen tumors had whole chromosomal gains only, while 27 tumors had either gains or losses. In 12 tumors, partial losses or gains were recorded. Statistical analysis did not show a significant effect of the presence of copy number alteration at any degree on recurrence or RFS (p=0.816, Figure 2). There was no association between any type of genetic alteration (*BRAF* fusion, *FGFR1* alteration, etc.) with the presence or extent of copy number loss or gain.

We have also analyzed impact of chromosomal losses and gains separately. Partial and/or whole chromosomal loss was detected in 23 patients; of which, 8 was whole chromosomal loss. Partial and/or whole chromosomal gain was observed in 34

**Table 6. Distribution of specific mutations across tumor locations**

| Mutation Type | Tumor Location | | | |
|---|---|---|---|---|
| | Supratentorial | Infratentorial | Spinal | Total |
| *BRAF* Alterations | 59 | 81 | 9 | 149 |
| *FGFR1* Alterations | 7 | 1 | 0 | 8 |
| *NF1* Alterations (inc. germline *NF1)* | 10 | 16 | 3 | 29 |
| Other MAPK Alterations | 7 | 1 | 0 | 8 |
| No abnormality found | 2 | 1 | 1 | 4 |

patients; of which 23 was whole chromosomal gains. Cases with any degree of chromosomal loss had a median RFS of 63 months whereas, it was 54 months for cases without any chromosomal losses. On the other hand, cases with chromosomal gain of any size had a median RFS of 63 months, whereas it was 54 months for cases without any chromosomal gains. No significant influence of whole chromosomal losses or gains was seen on recurrence, RFS and underlying molecular features of the tumor as well.

Previous studies proposed whole chromosomal gains as an age-associated alteration without an impact on outcome [45]. There were 14 cases with whole chromosomal gains with no other CNV. Thus, we excluded these cases and conducted the analysis on the remaining 25. The results showed no statistically significant difference between cases with or without CNV (excluding whole chromosomal gains).

## Impact of age on recurrence-free survival

The recurrence rates for patients younger than 14 years, between 14 and 21 years, and 21 years and over were 40.9%, 34.6%, and 20.4%, respectively. Although the recurrence rates are higher in the youngest age group, this difference was not statistically significant. (p=0.003).

Among patients who underwent subtotal resection, patients older than 21 years at diagnosis

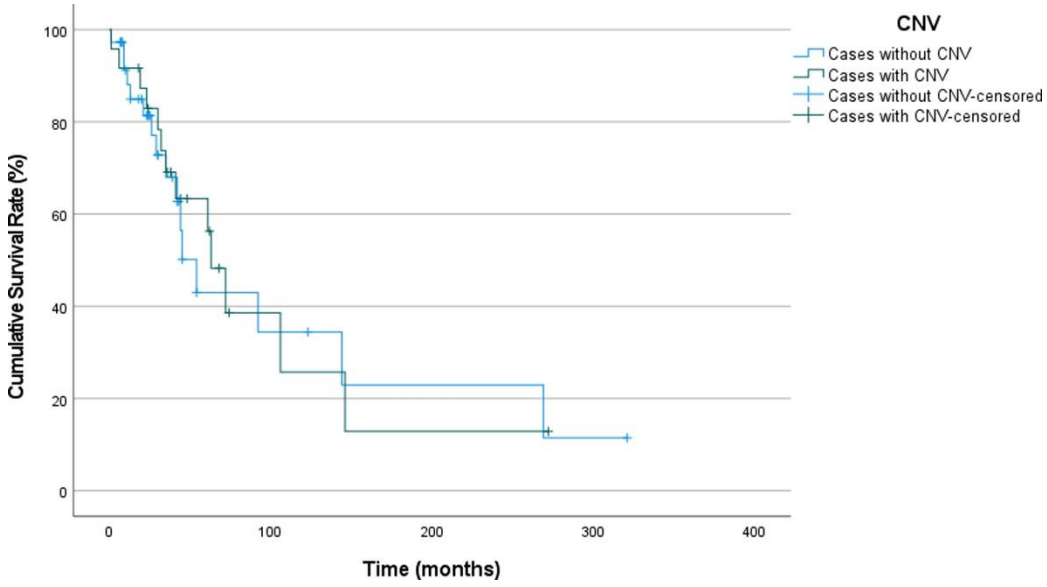


**Figure 2.** Recurrence-Free Survival in Patients with Pilocytic Astrocytoma, Stratified by Presence of Copy Number Variations (CNV: Copy number variation).

showed higher recurrence rates (p<0.001), while there was no difference between age groups among patients who underwent GTR. We further investigated the confounding factors on the difference in STR group and included tumor location; even after including tumor location as a variable, patients >21 years of age showed a significantly higher recurrence rate (p<0.001).

## Impact of extent of resection on recurrence-free survival

One-hundred-sixty-nine (33.9%) patients underwent GTR and 235 (47.1%) had STR. The data on the extent of surgery was missing in 95 (19.0%) cases. Recurrence rates were 8.5% and 20% for patients who underwent GTR and STR, respectively. The mean RFS was 321 months for patients who underwent GTR (CI: 292.3 – 350.1), and 160.9 months (CI:124.5 – 197.4) for patients who underwent STR. The patients with GTR have a significantly longer recurrence-free survival (RFS) compared to those with STR (log rank p <0.001, Figure 3). The median survival probability for overall survival could not be calculated due to absence of effect of extension of surgery on overall survival.

## Impact of chemotherapy and/or radiotherapy on recurrence-free survival

Among 81 patients who received chemotherapy, 77 had a prior STR. Patients who received chemotherapy demonstrated a shorter median RFS (141 vs 253 months). However, we think that this finding could be potentially skewed due to selection bias, as the patients with clinically more aggressive appearing tumors are more likely to receive chemotherapy. A great majority of cases (97.5%) received chemotherapy had subtotal resections, which strongly supports our hypothesis on selection bias. Thus, the observed difference in RFS may not be entirely surprising. A similar pattern was also observed among patients treated with radiotherapy (151 vs 243 months).

## Review of patients with neurofibromatosis 1

There were 24 confirmed neurofibromatosis Type 1 patients (11 female and 13 male), which comprise 4.8% of the entire cohort. Mean age at diagnosis was 19.5 years (3.1 – 42.7). Majority of the tumors (n=16) were in the posterior fossa, and four tumors were in the hypothalamic-suprachiasmatic region. Out of 15 patients with sufficient clinical data,

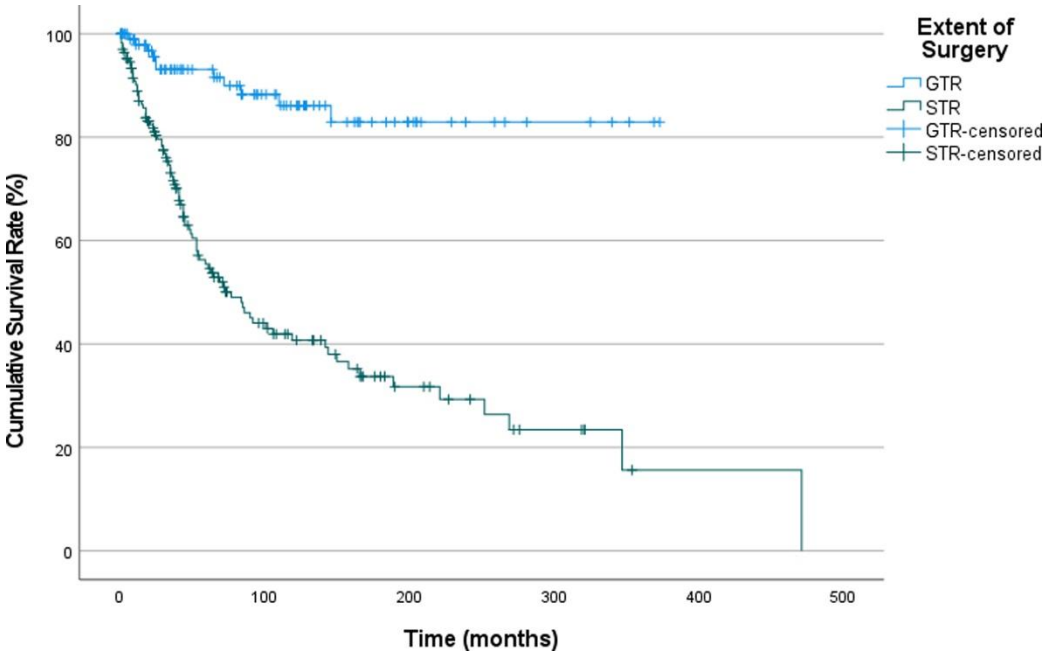


**Figure 3.** Recurrence-Free Survival in Patients with Pilocytic Astrocytoma, Stratified by the Extent of Surgical Resection (GTR: Gross total resection, STR: Subtotal resection).

three experienced recurrences. One patient died during follow-up period due to other causes (for details see table 1, case#454). Two cases had anaplastic features on histology, and the remaining 22 had classical PA morphology. None of the NF1-associated cases had mutations in other genes activating MAPK pathway (*BRAF, FGFR1*, etc.).

### Review of patients with >5-year follow-up

Given PAs grow slowly and sufficiently long follow-up times are crucial for a realistic assessment of outcome, we performed a subgroup analysis of the patients who have more than 5 years of follow-up (n=178). Mean follow up time in this subgroup was 263.3 months; median follow up time was 252 months. Out of the 164 patients in this group for whom we had adequate recurrence data, 68 experienced a recurrence. The type of surgery remained one of the strongest factors that determined the rate of recurrence and RFS, as cases with STR recurred more often and earlier (Figure 4). Sixty-one percent of the patients with STR experienced recurrence at least once whereas the recurrence rate was 11% for patients with GTR ($p<0.001$). We were not able to show an association between the underlying genetic alterations or tumor location and recurrence rate or RFS. The number of cases in each group was not sufficient to perform an analysis of RFS for cases with and without CNV.

After conducting a multivariate analysis of RFS considering the age groups, extent of resection, tumor location, NF1 status, and treatment modalities, we found that only the extent of resection had a statistically significant association with RFS (Table 7).

## Discussion

Multivariate analysis of outcome did not reveal any significant association with clinical and pathological variables except for extent of resection. Despite the large number of patients and follow-up time that is considerably longer than most studies in the literature, age, NF1 status, tumor location, adjuvant treatment and molecular alterations did not significantly influence RFS. These findings are at odds with some of the studies that suggest differences in outcome between adult and pediatric patients or between germline NF1 and wildtype tumors [46-48]. Some suggested that adult cases have a worse prognosis than pediatric counterparts [18-21] while others demonstrated a very favorable prognosis regardless of age [18, 49]. It is well recognized that older age is associated with a higher incidence of deaths compared to younger populations

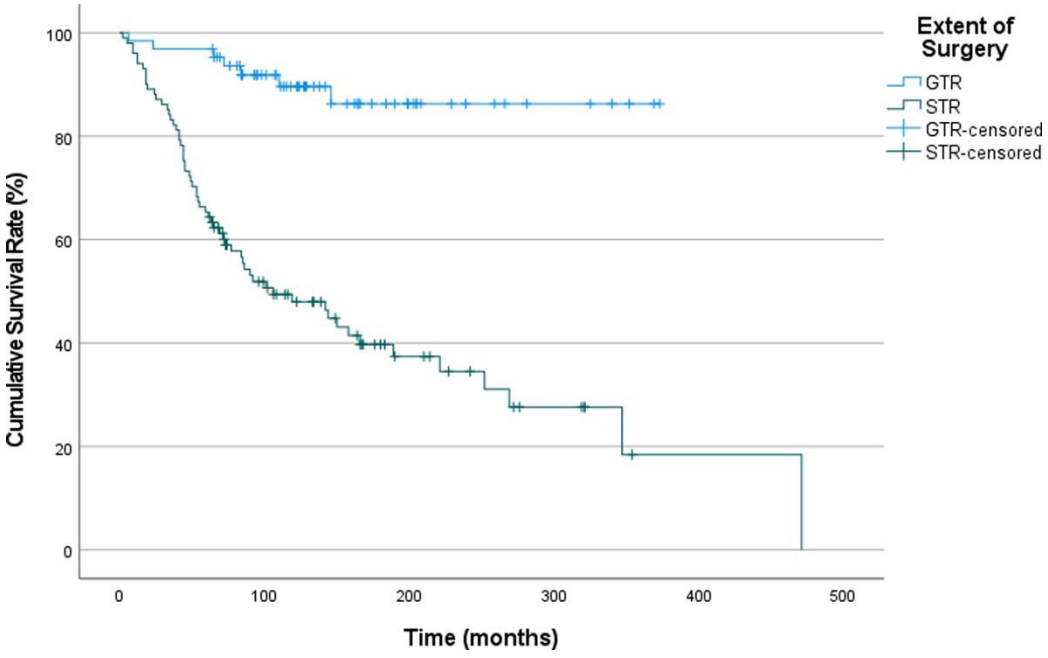


**Figure 4.** Subgroup Analysis of Recurrence-Free Survival in Patients with Pilocytic Astrocytoma Followed for ≥60 Months, Stratified by the Extent of Surgical Resection (GTR: Gross total resection, STR: Subtotal resection).

**Table 7. Multivariate analysis of factors for recurrence free survival probability**

| | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| **Variable** | Significance | HR | %95 CI | Significance | HR | %95 CI |
| Type of Surgery | | | | | | |
| *GTR vs STR* | <0.001 | 6.28 | 3.35 – 11.77 | <0.001 | 6.23 | 3.23 – 11.99 |
| | | | | | | |
| Location | 0.049 | | | 0.957 | | |
| *Infratentorial vs Supratentorial* | 0.027 | 1.58 | 1.05 – 2.36 | 0.925 | 0.98 | 0.63 – 1.51 |
| *Infratentorial vs Spinal* | 0.097 | 1.97 | 0.88 – 4.40 | 0.811 | 1.11 | 0.46 – 2.67 |
| | | | | | | |
| Age | 0.082 | | | 0.094 | | |
| *0-14 vs 14-21* | 0.564 | 0.855 | 0.50 – 1.46 | 0.337 | 0.76 | 0.44 – 1.33 |
| *0-14 vs >21* | 0.026 | 0.554 | 0.33 – 0.93 | 0.036 | 0.56 | 0.33 – 0.96 |
| | | | | | | |
| Sex | 0.266 | 1.25 | 0.85 – 1.83 | | | |

in patients with tumors. This is often attributed to accelerated epigenetic age and the simple effect on age on overall survival as opposed to factors directly associated with tumor progression [50, 51]. In our series, we have separated cases into three age groups and although recurrence rates increased with age, this difference was not significant. Even in subgroup analysis of cases with GTR and STR, age did not stand up as a significant factor. We were not able to assess PA specific overall survival because we observed no PA-related fatality.

It has been noted that location could be an independent prognostic variable and tumors located in the posterior fossa generally have more favorable outcomes [26, 52, 53]. This was presumed to be associated with surgical access to tumor and ability to remove completely, and therefore tumors located in regions difficult to access - specifically the brain stem and hypothalamo-suprachiasmatic region - were associated with worse outcomes [20, 54]. One particular study shows that while tumor location initially appears to be a strong prognosticator, its significance diminishes when considering the extent of the resection [55]. Our analysis failed to reveal a statistically significant relationship between location and recurrence rate or RFS, despite re-categorizing cases into narrower, and later, two broad groups - "hypothalamo-suprachiasmatic + brainstem" and "other locations".

The discrepancies between our findings and those in other studies may be due to the acceptance of univariate calculations as significant, even though they may not hold up on multivariate analyses when all pertinent variables, especially extent of resection, are considered. Another problem is the immediate acceptance of $p<0.05$ as a significant cut-off to determine biologically meaningful differences [56-58]. Yet, a series of 499 patients may still be insufficient to accurately determine the small but significant effect of some variables on outcome. One critical issue in our study is the absence of patients who died due to their disease progression and presence of rare deaths due to other factors. This could still reflect a limitation of sampling, since earlier studies reported occasional deaths due to disease progression in PA [59, 60]. The reported deaths may

obviously be associated with confounding factors (such as radiation treatment or pathological misclassification) or with the assumption that the death of a patient is always a consequence of disease progression.

Our data is sourced from a single referral center which minimizes certain confounding factors, allowing for a more streamlined analysis. However, the data are not associated with an epidemiologically relevant catchment area and may have selection bias due to the fact that our institution is a major national and international referral center. Therefore, our findings need further validation by large, and multi-institutional and epidemiologically relevant studies.

Influence of histological features on the course of the disease has always been a debate in the literature as well as the criteria for histological anaplasia. WHO defines PA with histologic features of anaplasia as PA with brisk mitotic activity with or without necrosis. While there exist a handful of studies suggesting an association between histological anaplasia in PA and unfavorable outcome [61, 62], our findings failed to provide substantial evidence to support or refute these claims. In our series, we had only 6 cases (among 499) with histologic features of anaplasia, a sample size insufficient for meaningful comparison. These six patients were adults, with a mean age of 46.2, ranging from 27 to 72 years old. Four tumors were located in posterior fossa whereas two were hemispheric. Of these cases, five tumors were analyzed by UCSF500 NGS platform and four of them revealed a *CDKN2A* loss. As stated in the result section, all four cases with *CDKN2A* loss had histological features of anaplasia. Although there seems to be an association between *CDKN2A* loss and histological features of anaplasia, small number of cases prevent a meaningful and statistically significant conclusion.

In our series, we identified 6 cases of PMA, which suggests that the prevalence of PMA among pilocytic tumors is only around 1%, also supported by earlier studies [29, 63]. The overall ratio and diagnostic criteria of PMA and PA with anaplastic features have been quite variable, and some published studies reported a rate of 8-20% of PMA among their PA cases [63-65]. This may reflect the challenges of making the diagnosis or the variations in histological criteria for PMA. We believe that PMA is typically a tumor of young age and hypothalamic/chiasmatic location with monomorphous histological features and deviations from this typical spectrum should be interpreted with caution [63, 65]. This could be the reason some studies did not identify significant prognostic differences between tumors designated as PMA and PA [64, 66].

Molecular studies have consistently demonstrated activation of the MAPK pathway in all cases of PA. The most prevalent genetic alteration involves kinase domain duplication and fusion of the *BRAF* gene with KIAA1549. Other alterations seen in PA are *BRAF*V600E mutations, *FGFR1* alterations, NF1 alterations and also rare alterations in other components of MAPK pathway. *FGFR1* alterations are highly enriched in supratentorial tumors, but spinal and cerebellar tumors also showed this molecular alteration, as observed in previous series [33, 67]. Many reports showed evidence that molecular features such as *TERT* promoter mutation, *CDKN2A* loss, *TP53* mutation, or chromosomal copy number alterations may be associated with adverse clinical outcome [68-71]. In our series, we were unable to identify a meaningful association between molecular alterations and RFS. Whole or partial chromosome copy number alterations could not be correlated with outcome either. This was partially due to small numbers and partly due to short follow-up times for tumors with comprehensive molecular analysis since such analyses began only in recent years. Analysis of impact of molecular alterations including copy number variations are more likely to provide meaningful results in future studies with longer follow-up terms and larger number of patients. While molecular alterations help in establishing the diagnosis in PA, their association with location and outcome remain tenuous and require larger and prospective studies for definitive conclusions.

One other point of discussion that has been a matter of contention is the decision to use the term “tumor type” instead of “tumor entity” by WHO 2021 classification. While this seems like a reasonable change prima facia due to the desire to classify tumors similar to animal and plant kingdom taxonomy, this approach entirely misses the point that tumors do not fit into neat categories of species and

genera that could be easily placed in a taxonomical framework similar to animals and plants. The major objection to the use of tumor "type" is that a diagnostic entity such as PA may not be composed of a single tumor "type" but rather is a group of tumors that have sufficiently similar clinicopathological (including genetic) features to constitute a meaningful group of disorders from the perspective of the treating physician. Attempting to create "types" with every advancing bit of information would not result in the same clinically meaningful group of diseases. This is demonstrated in our study that despite their excellent outcome, tumors classified as PA harbor sufficiently different genetic alterations, radiological and pathological features that are not sufficient enough to consider them as belonging to a different entity but may arguably imply different tumor "types" or "subtypes". In the opinion of the authors, attempting to classify tumors by placing rigid types and subtypes akin to genera, and species designations for animal and plant kingdoms may underestimate the biological diversity of pathological processes as opposed to evolutionary biology. This approach also ignores the principal necessity of trying to classify tumors to be able to manage the patients successfully. From that perspective, the tumors in this series that we believe belong to PA as an entity, have excellent long-term survival and may be considered "chronic diseases" and managed accordingly [72, 73].

Our study is in agreement with many of the prior studies highlighting the extent of resection as the key determinant of recurrence in PA [16, 53, 55, 74-76]. PA still remains a predominantly surgical disease due to the importance of extent of resection and every attempt should be made to achieve GTR for maximum benefit [77, 78]. But, there is yet much to be learned about PA, especially the biological implication of the histological features of anaplasia and additional molecular abnormalities. We were unable to identify any association between these variables and outcome, most likely because we began analyzing some of these molecular alterations only recently and sufficient time has not passed to see their effect on outcome. Prospective studies with follow up times longer than 10 years will be necessary to accurately determine the significance of molecular alterations or other variables. This will require collaborative efforts and creating tumor registries that provide platforms for longitudinal studies.

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