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Histopathologic review of pineal parenchymal tumors identifies novel morphologic subtypes and prognostic factors for outcome

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Abstract

Background. Pineal parenchymal tumors (PPTs) are rare neoplasms of the central nervous system, and data concerning clinical outcomes are limited. The purpose of this study was to define the clinical behavior of PPT according to current histopathologic criteria and identify prognostic factors to guide therapeutic decisions.

Methods. Seventy-five patients treated for PPT at a single institution between 1992 and 2015 were retrospectively identified. Forty-five resection specimens were available and re-reviewed. Freedom from progression (FFP) and overall survival (OS) were estimated using the Kaplan-Meier method and compared using log-rank tests.

Results. Median follow-up was 4.1 years. All patients initially underwent surgery; 78% of patients with PPT of intermediate differentiation (PPTID) and all patients with pineoblastoma received adjuvant therapy. Pathologic re-review refined classification in 27% of cases, with the majority of these being adult patients with pineal tumors originally classified as pineoblastomas that more accurately resembled PPTID based on the 2007 WHO classification.

Classification. Our histologic review also identified that PPTIDs can be classified into small-cell and large-cell morphologic subtypes, which have distinct clinical outcomes. Tumor grade, extent of resection, and neuraxis spread were prognostic for FFP. PPTID subtype, extent of resection, and neuraxis spread were prognostic for OS. Genetic analysis of a pineoblastoma case identified somatic mutations of *DICER1*, *ARID1A*, and *KDM5C* genes.

Conclusions. PPTIDs can be classified into 1 of 2 novel morphologic subtypes that are associated with distinct clinical outcomes. Tumor grade, neuraxis spread, and extent of resection also influence outcome for patients with PPT.

Key words:

DICER1 | pineal parenchymal tumor | pineal parenchymal tumor of intermediate differentiation | pineoblastoma | pineocytoma

The pineal body is an endocrine gland within the epithalamus that modulates circadian rhythms through the production of melatonin.[1] Tumors affecting the pineal gland are uncommon and account for <0.5% of all intracranial malignancies.[2] Despite their rarity, a large spectrum of neoplasms can arise in the vicinity of the pineal

gland or spread to the pineal region including germ cell tumors, gliomas, papillary tumor of the pineal region, and metastases. Primary neuronal tumors arising intrinsically within the pineal gland are referred to as pineal parenchymal tumors (PPTs). The most recent World Health Organization (WHO) Classification of Tumors of the Central Nervous System from 2007 categorizes PPT as pineocytomas (grade I), pineal parenchymal tumors of intermediate differentiation (PPTID, grade II or grade III), or pineoblastomas (grade IV).[3] Surgery is essential for all pineal tumors in order to obtain a tissue diagnosis and often to alleviate symptoms from impaired cerebrospinal fluid flow from the third ventricle into the cerebral aqueduct.[4] Despite their common origin and the importance of surgical resection, the epidemiology and treatment of PPTs are diverse. Classically, pineoblastomas arise in pediatric patients, whereas pineocytomas and PPTIDs typically occur later in life.[5] Pineocytomas have a favorable prognosis, with 5-year survival approaching 90% following gross total resection (GTR).[6] Conversely, pineoblastomas, which have a high rate of recurrence and a propensity for neuraxial spread, are often managed with adjuvant craniospinal irradiation and multi-agent chemotherapy.[7] PPTID is an intermediate entity, with histologic features falling between well-differentiated pineocytomas and undifferentiated pineoblastomas.

Given the relative rarity of PPTs in general, and PPTIDs in particular, outcomes data are largely limited to retrospective case series. The largest primary report of PPT outcomes compiled data documented 76 patients in 12 European centers treated from 1972 to 1997.[8] This study demonstrated 5-year survival of 91%, 74%, 39%, and 10% for grades I–IV neoplasms, respectively, but relied on diagnostic criteria that have since been revised. Tumor diameter <2.5 cm and low-grade histology were found to be prognostic for outcome, but extent of resection and radiotherapy had no clear influence on survival. Shortly thereafter, a pooled analysis of 37 previously published cases with 64 newly reported patients identified metastases at diagnosis, high-grade histology, and subtotal resection (STR) as variables that negatively influenced overall survival (OS) for PPTID and pineoblastoma.[7] Most recently, a systematic review of 64 studies consisting of 168 pineocytoma patients demonstrated that 5-year OS for low-grade PPT decreased precipitously in patients who received STR followed by radiotherapy (17%) versus GTR without adjuvant therapy (88%).[9] Beyond these reports, the majority of literature pertaining to PPT consists of retrospective case series from single institutions with few patients and short follow-up.[10–33] Furthermore, the updated diagnostic classification of PPT in the 2007 release of the WHO Classification of Tumors of Central Nervous System renders interpretation of pathophysiologic and outcome data from prior reports challenging.[3] We therefore sought to characterize outcomes and patterns of failure for PPT according to the latest histopathologic criteria as well as identify prognostic factors to guide therapeutic decisions. Toward that end, 75 patients with pineal region tumors treated at a single institution from 1992 to 2015 were retrospectively identified, and all available resection specimens (n = 45) were re-reviewed centrally. The data from this cohort, which is one of the largest groups of patients with PPT according to current diagnostic criteria published to date, reveal new insights about the epidemiology, histopathology, clinical behavior, and optimal treatment of PPT.
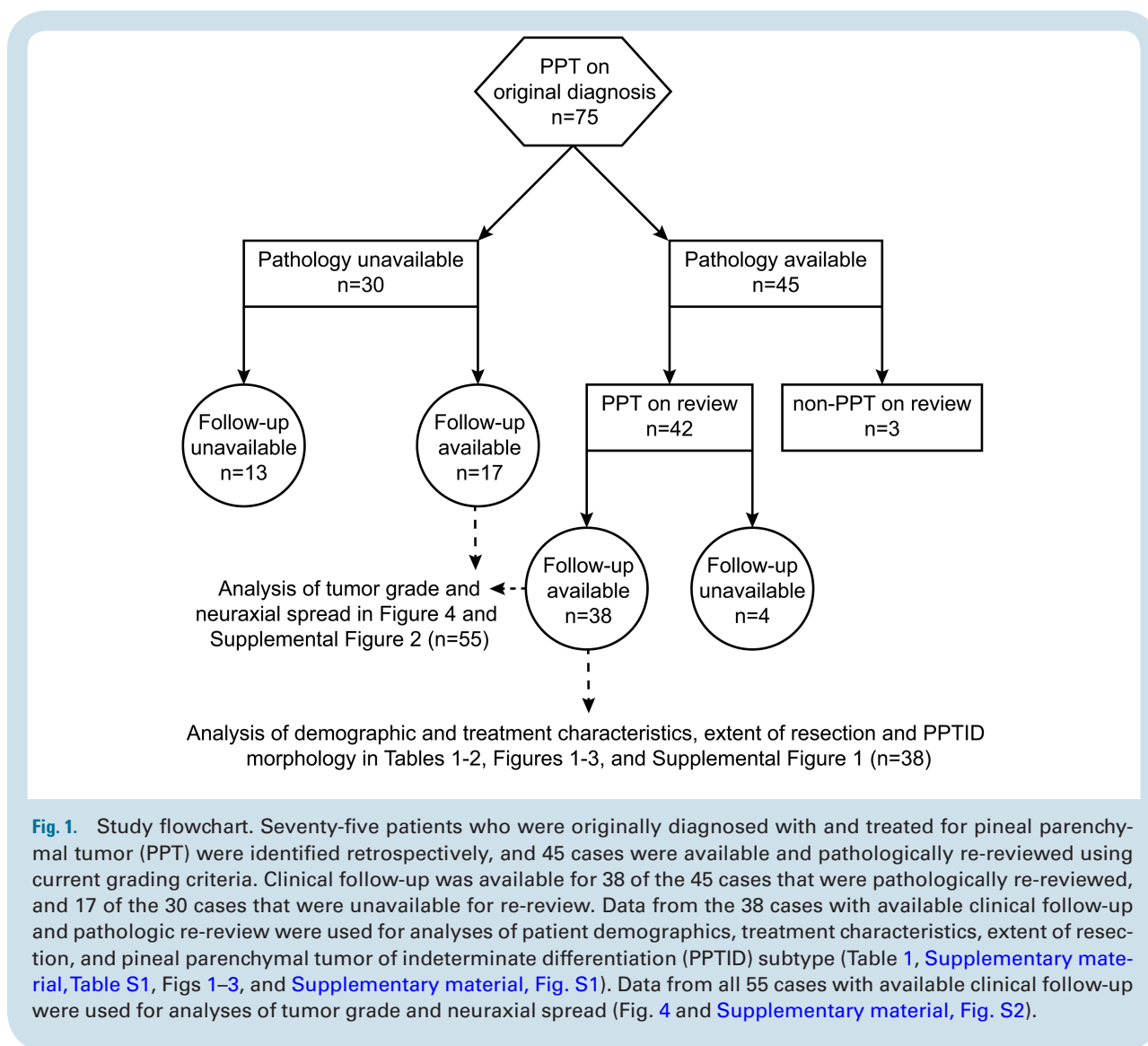
Materials and Methods

Pathologic Review

Seventy-five patients treated for a diagnosis of PPT at the University of California San Francisco between June 1988 and August 2015 were retrospectively identified for this analysis, which was approved by the Institutional Review Board. Forty-five cases from July 1992 to August 2015 were available and were pathologically re-evaluated by 2 neuropathologists (D.A.S. and T.T.) based on grading criteria from the 2007 WHO Classification of Tumors of the Central Nervous System[3] (Fig. 1). Both neuropathologists agreed on the diagnosis for all cases without interobserver discordance. This led to reclassification of diagnoses in 12 instances (27% of cases), all but one of which were diagnosed before 2007. Nine tumors that were previously classified as pineoblastoma in adults were revised to PPTID (Supplementary material, Table S1). Two tumors in adult patients that were initially classified as PPT were determined to most likely represent metastases (one from pulmonary small-cell carcinoma and the other a malignant melanoma with small-cell features). The last case was a pineal region tumor in a 20 year old man that had been previously classified as "malignant neoplasm most compatible with pineoblastoma, WHO grade IV" but which lacked the typical histologic features of pineoblastoma such as Flexner-Wintersteiner rosettes or the usual small blue cell appearance and had an atypical immunohistochemical profile including positivity for CD117 (c-Kit). As such these 3 cases, which were thought not to definitely represent PPT on re-evaluation, were excluded from all subsequent analyses (Fig. 1). Outcomes data from 17 additional patients originally treated for PPT according to diagnostic criteria from their respective eras, but for whom resection specimens were not available, were added to the clinical data from pathologically re-reviewed cases to sufficiently power survival analysis according to histology or neuraxial spread (Figs 1 and 4).

Immunhistochemical Staining

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections at the UCSF Immunohistochemistry Laboratory. Primary antibodies used were as follows: synaptophysin (polyclonal, Cell Marque, 1:100 dilution, ER2 antigen retrieval buffer), neurofilament (clone 2F11, Cell Marque, undiluted, ER1 antigen retrieval buffer), NeuN (clone A60, Chemicon, 1:4000 dilution, ER1 antigen retrieval buffer), MAP2 (clone HM2, Sigma, 1:20 000 dilution, ER1 antigen retrieval buffer), GFAP (polyclonal, Dako, 1:300 dilution, no antigen retrieval), and OLIG2 (polyclonal, Immuno Bio Labs, 1:200 dilution, ER1 antigen retrieval buffer). All staining was performed in a Leica Bond 3 automated staining processor following antigen retrieval in the indicated buffer and Ventana ultraView Universal DAB detection.

Fig. 1. Study flowchart. Seventy-five patients who were originally diagnosed with and treated for pineal parenchymal tumor (PPT) were identified retrospectively, and 45 cases were available and pathologically re-reviewed using current grading criteria. Clinical follow-up was available for 38 of the 45 cases that were pathologically re-reviewed, and 17 of the 30 cases that were unavailable for re-review. Data from the 38 cases with available clinical follow-up and pathologic re-review were used for analyses of patient demographics, treatment characteristics, extent of resection, and pineal parenchymal tumor of indeterminate differentiation (PPTID) subtype (Table 1, Supplementary material, Table S1, Figs 1–3, and Supplementary material, Fig. S1). Data from all 55 cases with available clinical follow-up were used for analyses of tumor grade and neuraxial spread (Fig. 4 and Supplementary material, Fig. S2).

Clinical Outcomes Assessments

Demographic and clinical follow-up data were extracted from the medical records and institutional cancer registry for all cases. Unless specified otherwise, all statistical analyses were performed on 38 pathologically confirmed cases of PPT with available follow-up data using the revised histologic grade when applicable. The length of follow-up was defined as the duration of time from each patient's most definitive surgery to the date of recurrence for freedom from progression (FFP), the date of death for OS, or the most recent clinical or radiographic evaluation for those who experienced neither recurrence nor death.

Age at diagnosis (continuous variable) was compared with revised diagnosis categorized as pineocytoma, PPTID grade II, PPTID grade III, pineoblastoma (group variable), and PPTID subtype (small cell vs large cell) using ANOVA. Age according to tumor grade and morphology are presented as median (with range) and mean±standard deviation. The association between extent of resection (as

defined on imaging) and tumor grade was assessed using a chi-square test. To examine the relationships between extent of resection, neuraxis spread at diagnosis, adjuvant therapy, disease control, and death, Fisher exact tests for small sample sizes were reported. FFP and OS were estimated using the Kaplan-Meier (KM) method. FFP and OS patterns by KM for GTR status, PPTID subtype, tumor grade, and neuraxis spread were assessed using the log-rank test or log-rank test for trend, as indicated. No sample size calculation was performed for this exploratory analysis. Two-sided P values were reported, and P values <.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism 6 and SAS v9.4.

Targeted Next-generation Sequencing

DNA was extracted from a peripheral blood sample and formalin-fixed, paraffin-embedded tumor tissue using Qiagen DNA extraction kits. Capture-based next-generation

sequencing was performed at the UCSF Clinical Cancer Genomics Laboratory using an assay that targets the coding regions of 510 cancer-related genes, select introns from 40 genes, and TERT promoter with a total sequencing footprint of 2.8 Mb (UCSF500 panel). Target enrichment was performed by hybrid capture using a custom oligonucleotide library. Sequencing was performed on an Illumina HiSeq 2500. Bioinformatics analysis to assess for somatic single nucleotide variants, small insertions/deletions, structural variants, and copy number changes was performed using the following software packages: BWA: 0.7.10-r789, Samtools: 1.1 (using htslib 1.1), Picard tools: 1.97 (1504), GATK: 2014.4-3.3.0-0-ga3711, CNVkit: 0.3.3, Pindel: 0.2.5a7, SATK: 2013.1-10- gd6fa6c3, Annovar: v2015Mar22, Freebayes: 0.9.20 and Delly: 0.5, CNVkit, and Nexus Copy Number.

Results

Patient, diagnosis, and treatment characteristics according to revised histopathologic evaluation are presented in Table 1. The median age at diagnosis for all patients was 32.4 years (range: 3.3–64.8 y). A diagnosis of pineoblastoma was made in significantly younger patients compared with other tumor grades (median: 8.9 y; mean: 6.3 ± 5.6 y; P = .0002) (Supplementary material, Fig. S1A). Fifty-five percent of cases were male, but the association between sex and PPT incidence was not significant (Table 1). The most common presenting symptoms were headache (76%), nausea and vomiting (45%), ataxia (45%), vision changes (32%), and altered mental status (29%). Three patients were found to have disseminated disease at diagnosis either by cerebrospinal fluid (CSF) cytology or imaging, neither of which was associated with disease control or death.

With respect to treatment, GTR was achieved in 50% of patients, with STR in 34%, and biopsy alone in 16% (Table 1). There were no differences in the ability to achieve GTR according to histology. No tumors recurred following GTR, which was significantly associated with both disease control (P = .04) and survival (P = .04) (Fig. 2 A and B). Although the number of cases failed to meet the threshold for robust outcomes analyses according to extent of resection by tumor grade, there was a trend toward improved survival with GTR for PPTID grade III tumors (P = .14). Patients with PPTID and pineoblastoma were significantly more likely to receive adjuvant radiation (P < .0001) and/or chemotherapy (P = .0001) than those with pineocytoma (Table 1). The vast majority of those who received radiation were treated at the entire craniospinal axis to a median dose of 36 Gy, followed by a fractionated boost to the pineal gland for a median total dose of 55.8 Gy. Four of the 6 individuals with PPTID grade II treated with craniospinal irradiation were initially diagnosed with pineoblastoma. Both patients treated with surgery followed by focal radiation were initially diagnosed with PPTID grade II but had evidence of residual local disease. Most patients who received chemotherapy were treated with a combination of platinum-based (94%; cisplatin or carboplatin) and alkylating agents (83%; cyclophosphamide, lomustine, procarbazine, or ifosfamide). The microtubule poison vincristine (67%) and the topoisomerase inhibitor etoposide (17%) were also common. Despite the association between adjuvant therapy and higher tumor grade, patients who received chemotherapy and/or radiation were no more likely to experience disease recurrence or death than those who did not.

The median overall follow-up was 4.1 years (range: 4 d–15.2 y), during which there were 4 tumor recurrences and 7 deaths (Table 1). On long-term follow-up, 11 patients had persistent side effects from treatment including growth defects, endocrine dysfunction, infertility, and cognitive deficits in 3 patients who were treated as children. The median time to tumor recurrence was 3.5 years (range: 1.1–8.5 y), which was longer than the median time to death (1.1 years; range: 4 d– 8.5 y) due to 2 deaths from perioperative complications without evidence of disease progression. Importantly, none of these were due to air embolism, as might be expected from surgery in the seated position.

Table 1. Demographic, disease, and treatment characteristics of pineal parenchymal tumors according to revised histology

	Pineocytoma	PPTID Grade II	PPTID Grade III	Pineoblastoma
Number	13	10 (6 small cell, 4 large cell)	8 (5 small cell, 3 large cell)	7
Median age at diagnosis (y) [range]	33 [12.8–61]	41 [25.4–51.8]	31.7 [9.6–64.8]	6.3 [3.3–17.5]
Female-to-male ratio	1:1.8	1:0.7	1:3	1:1.3
Neuraxis spread	0 (0%)	1 (10%)	1 (12.5%)	1 (14%)
GTR	9 (69%)	4 (40%)	4 (50%)	4 (57%)
Chemotherapy	0 (0%)	6 (60%)	5 (63%)	7 (100%)
Radiation	0 (0%)	8 (80%)	6 (75%)	6 (86%)
Pineal region only	0 (0%)	2 (20%)	0 (0%)	0 (0%)
CSI + pineal boost	0 (0%)	6 (80%)	6 (75%)	6 (86%)
Disease recurrence[a]	0 (0%)	1 (10%)	2 (25%)	1 (14%)
Death	2 (15%)	1 (10%)	3 (37.5%)	1 (14%)

Abbreviations: CSI, craniospinal irradiation; GTR, gross total resection; PPTID, pineal parenchymal tumor of intermediate differentiation; y, year.
[a]PPTID grade III and pineoblastoma recurrences occurred simultaneously in the pineal region and distantly within the neuraxis; the one PPTID grade II recurrence was limited to the pineal region.
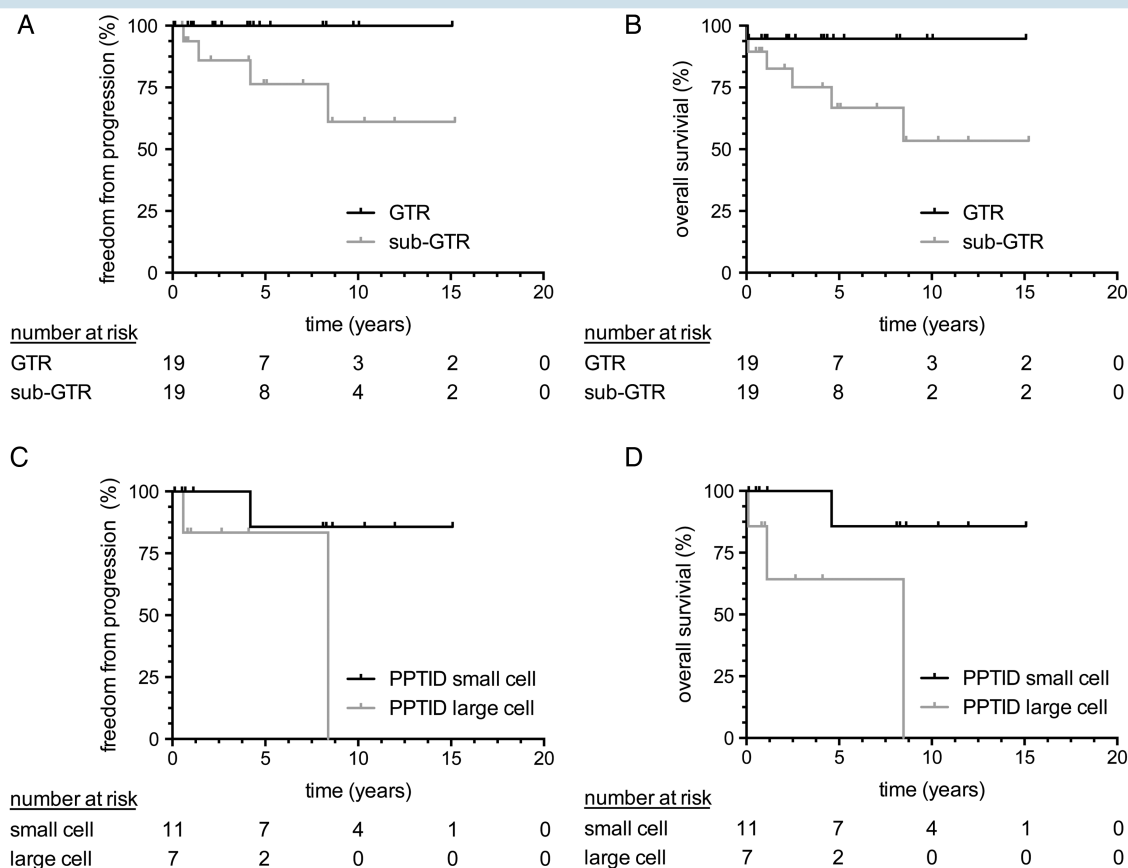
Fig. 2. Pineal parenchymal tumor (PPT) outcomes according to revised histology. (A, B) For all patients regardless of tumor grade, gross total resection (GTR) is associated with improved disease control (P = .04) and improved overall survival (P = .04) by Fisher exact test when compared with subtotal resection and biopsy (sub-GTR). (C, D) For patients with PPTs of indeterminate differentiation (PPTIDs), small-cell morphology is associated with improved overall survival (P = .02) and a trend toward improved progression-free survival (P = .07) by log-rank test.

Salvage therapy included re-irradiation in 1 case and chemotherapy in 3 cases. All disease recurrences of PPTID grade III and pineoblastoma occurred simultaneously in the pineal region and distantly within the neuraxis, whereas the lone PPTID grade II recurrence was limited to the pineal region. All patients who recurred ultimately died from progressive disease, and recurrence was negatively associated with survival ($P < .0001$). Notably, both deaths in the pineocytoma group occurred in the immediate postoperative period and were attributed to pulmonary embolism and intracranial hemorrhage with sepsis, respectively.

Pathologic review of this series of PPT identified 18 tumors that were classified as PPTID (Table 1). These tumors lacked the well-differentiated tumor cells resembling pineocytes with frequent pineocytomatous rosettes that were observed in pineocytomas, but they also lacked the sheets of primitive small blue cells with frequent mitoses, apoptotic bodies, nuclear molding, and necrosis that were observed in pineoblastomas. We observed tumors with 2 distinct morphologic appearances within this cohort of PPTIDs, which we heretofore describe as the "large-cell" and "small-cell" subtypes of PPTID (Fig. 3). PPTIDs corresponding to the large-cell subtype were characterized by larger nuclei with vesicular chromatin

pattern, greater nuclear pleomorphism, ample cytoplasm, and abundant neuropil-like stroma in the background. In contrast, PPTIDs corresponding to the small-cell subtype had smaller, more uniform nuclei with scant cytoplasm and very little stroma in the background. The tumor cells in the small-cell subtype were present in diffuse sheets and had inconspicuous nucleoli and lacked the nuclear pleomorphism, nuclear molding, apoptotic debris, and significant mitotic activity characteristic of pineoblastomas. We noted that the tumors we classified as the small-cell subtype of PPTID had significant histologic overlap with central neurocytomas, which typically arise in the lateral ventricles but lack the neurocytic rosettes sometimes seen in neurocytomas. Immunostaining patterns in our cohort of PPTD were similar to those that were reported previously.[12] Specifically, positivity for synaptophysin and neurofilament staining was present in all PPTs, with diffuse strong staining in all pineocytomas and variable staining in PPTIDs and pineoblastomas ranging from focal labeling of clusters of cells to diffuse strong staining in most tumor cells. Immunostaining profile was not found to be useful for differentiating between the small-cell and large-cell subtypes of PPTID, although the more abundant neuropil-like stroma in the large-cell subtype was noted to result
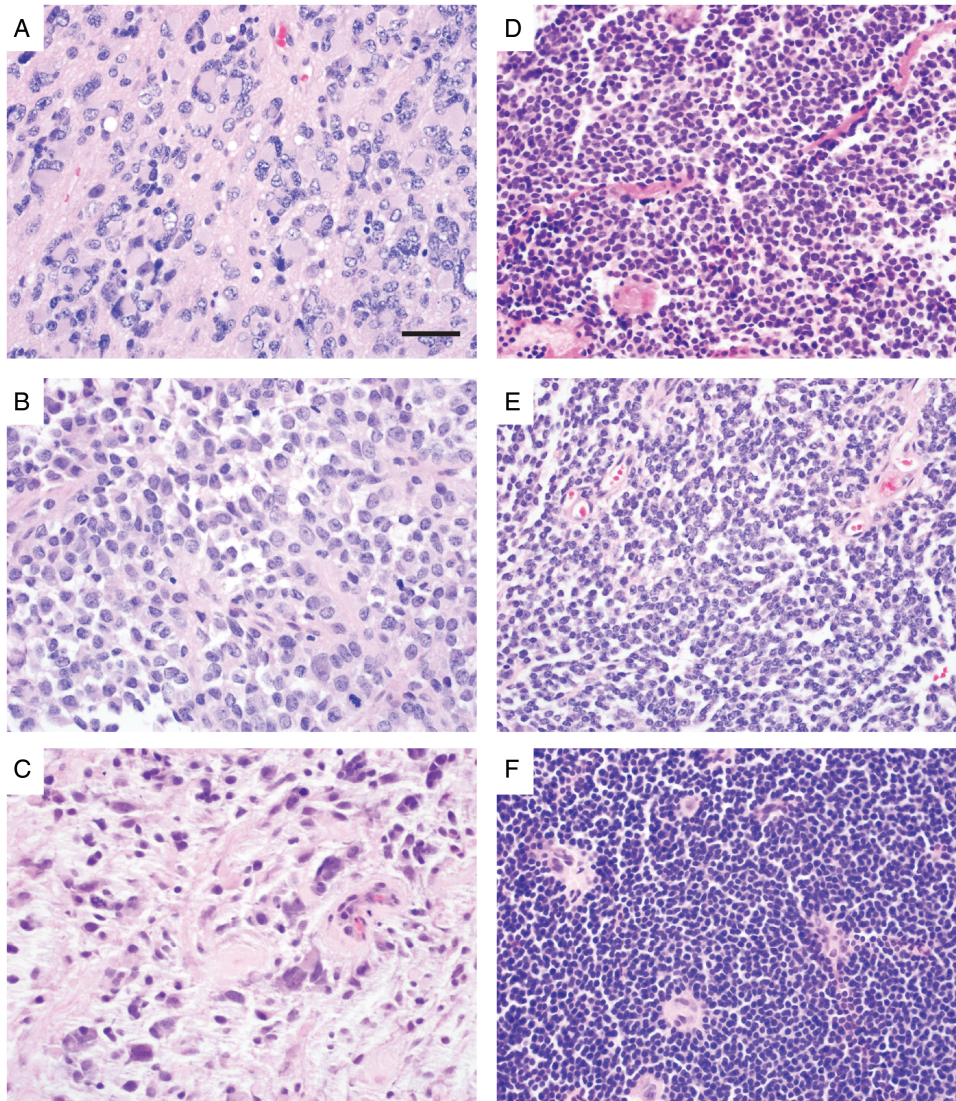
Fig. 3. Pathologic review of pineal parenchymal tumors of intermediate differentiation (PPTIDs) identifies 2 distinct morphologic subtypes: large-cell and small-cell. (A–C) H&E stained sections of PPTID cases corresponding to the large-cell subtype, which have larger nuclei with greater nuclear pleomorphism, more abundant cytoplasm, and more abundant neurophil-like stroma in the background. (D–F) H&E stained sections of PPTID cases corresponding to the small-cell subtype, which have smaller, more uniform nuclei with inconspicuous nucleoli and scant cytoplasm with very little stroma in the background. Scale bar, 20 μm.

in more robust neurofilament staining (Supplementary material, Fig. S3 and Supplementary material, Table S2). In contrast to the NeuN positivity reported in most central neurocytomas,[34] immunostaining for NeuN was absent in the 2 PPTID cases assessed (Supplementary material, Fig. S3 and Supplementary material, Table S2).

Among the 18 PPTIDs in this series, 7 corresponded to the large-cell subtype and 11 corresponded to the small-cell subtype. Based on the 2007 WHO Classification grading criteria using a mitotic cutoff of 6 mitoses per 10 high-power fields, 4 of the large-cell PPTIDs were best classified as grade II and 3 as grade III, whereas 6 of the small-cell PPTIDs were classified as grade II and 5 as grade III (Table 1).[3] There was no difference in the age distribution of patients with small- or large-cell subtypes of PPTID

(Supplementary material, Fig. S1B). However, small-cell tumors were associated with improved OS (P = .02) and demonstrated a trend toward improved FFP (P = .07) as compared with large-cell subtypes (Fig 2 C and D).

The number of pathologically re-reviewed cases with available clinical follow-up failed to meet the threshold for survival analysis according to histology or neuraxis spread. Therefore, we extracted outcomes data from 17 additional patients originally diagnosed and treated for PPT for whom resection specimens were not available for pathologic re-review (5 pineocytoma, 12 pineoblastoma) (Fig. 1). Data from PPTID cases were pooled, and a secondary statistical analysis was performed in combination with the aforementioned 38 cases. The median overall follow-up in this pooled cohort was 3.1 years (range: 4 d– 23.0 y), and the
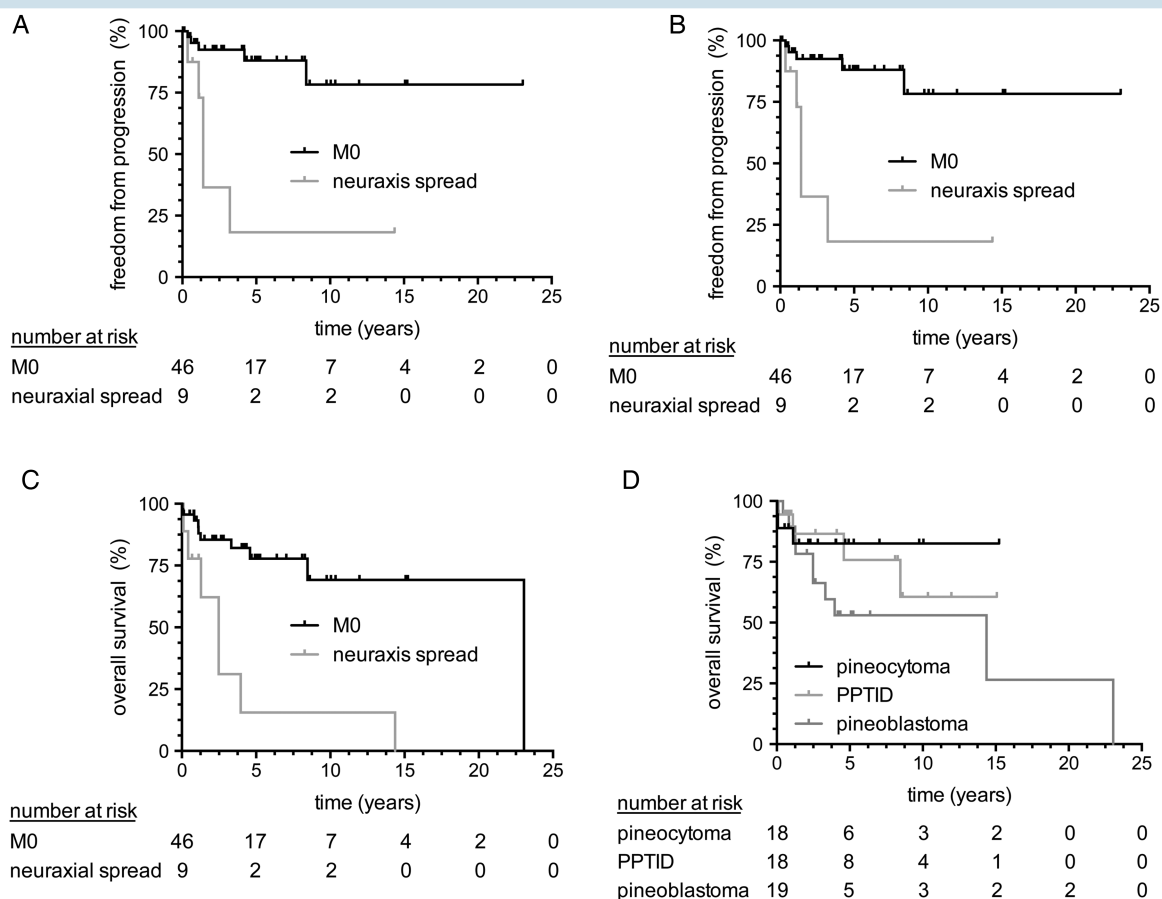
Fig. 4. Pineal parenchymal tumor (PPT) outcomes from a pooled cohort of cases with and without pathologic re-review. (A, B) Tumor histology (P = .03) and neuraxis spread (P = .001) are significantly associated with freedom from progression by log-rank test. (B, C) Neuraxis spread at diagnosis is prognostic for overall survival (OS) by log-rank test (P = .0003), and tumor histology shows a trend for OS by log-rank test for trend (P = .11). Of note, one patient treated with GTR and adjuvant chemoradiation for pineoblastoma without evidence of neuraxis spread at diagnosis died after 23 years of follow-up from a secondary high-grade sarcoma of the lumbar spine. The surgical specimen for this patient could not be located for pathologic re-review.

age distribution and relationship between GTR and survival paralleled the findings in pathologically re-reviewed cases (Supplementary material, Fig. S1). Importantly, the number of cases with neuraxis spread (n = 9; PPTID: 3, pineoblastoma: 6), tumor recurrences (n = 9; PPTID: 2, pineoblastoma: 7), and deaths (n = 17; pineocytoma: 3, PPTID: 4, pineoblastoma: 10) provided us with sufficient power for survival analysis. According to this approach, 5- and 10-year FFP probabilities were 100% and 100% for pineocytoma, 82% and 65% for PPTID, and 58% and 58% for pineoblastoma. Both tumor histology (P = .03) and neuraxis spread at diagnosis (P = .001) were significantly associated with FFP by log-rank test (Fig. 4 A and B). Five- and 10-year OS probabilities were 83% and 83% for pineocytoma, 76% and 61% for PPTID, and 53% and 26% for pineoblastoma. Neuraxis spread was prognostic for OS by log-rank test (P = .0003), and there was impaired survival with high-grade tumor histology by log-rank test for a trend that failed to reach statistical significance (P = .11) (Fig. 4 C and D).

At present, the genetic alterations that drive PPTs are largely unknown except for the subset of pineoblastomas

that arise in patients with germline mutations in either *RB1* or *DICER1* as part of the retinoblastoma and *DICER1* tumor-predisposition syndromes (OMIM 180200 and 606241). In order to better understand the molecular pathogenesis of PPTs and identify biomarkers that might aid classification among the different tumor entities, we performed targeted next-generation sequencing of 510 cancer-associated genes on genomic DNA isolated from an 18 month-old boy who underwent resection of a large, avidly enhancing tumor centered in the midline of the brain within the pineal region and cerebellar vermis (Fig. 5 A and B). Histologic sections from this tumor demonstrated a highly cellular, primitive, small round blue cell tumor with neuronal differentiation as evidenced by synaptophysin immunostaining (Fig. 5 C). Although a diagnosis of "medulloblastoma, WHO grade IV" was made given the presumed anatomic site of origin in the cerebellar vermis, subsequent next-generation sequencing did not identify any mutations or cytogenetic changes that are typical of medulloblastoma. Instead, 2 inactivating mutations in *DICER1*, a frameshift mutation in *ARID1A*, and a missense mutation in *KDM5C*
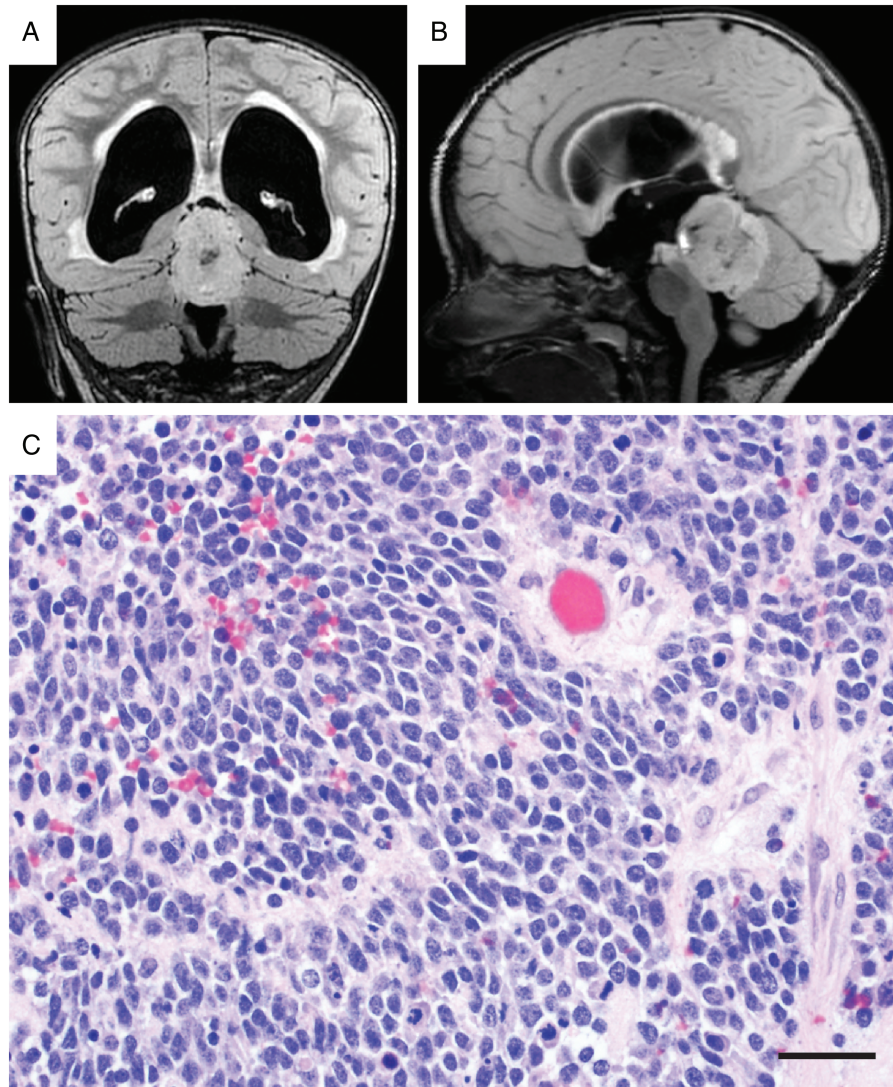
Gene	Variant	Transcript ID	Blood MAF	Tumor MAF
DICER1	p.V1080fs	NM_030621	0%	42%
DICER1	p.E1813D	NM_030621	0%	46%
ARID1A	p.G370fs	NM_006015	0%	58%
KDM5C	p.Y525D	NM_004187	0%	99%

Fig. 5. Genomic analysis of a pineoblastoma arising in a young pediatric patient identifies novel somatic mutations involving *DICER1*, *ARID1A*, and *KDM5C*. (A) Coronal T1 post-gadolinium and (B) sagittal fluid-attenuated inversion recovery MR imaging of an 18 month-old boy demonstrating a large, avidly enhancing mass centered in the midline within the region of the pineal gland and causing compression of the subjacent cerebellar vermis. (C) H&E stained section of the tumor showing a primitive small round blue cell neoplasm arranged in sheets with nuclear molding, numerous mitotic figures, and apoptotic debris. Scale bar, 20 µm. (D) List of somatic mutations that were identified upon targeted next-generation sequencing of 510 cancer-associated genes on genomic DNA isolated from peripheral blood and tumor tissue. MAF, mutant allele frequency.

were found in the tumor but were not present in constitutional DNA isolated from this patient's blood (Fig. 5 D). Re-consideration of the anatomic site of origin of this tumor favored the pineal gland over the cerebellar vermis. An amended pathologic diagnosis of "primitive small blue cell tumor most consistent with pineoblastoma, WHO grade IV" was made. At present, there are no reliable morphologic or immunophenotypic methods for distinguishing medulloblastoma and pineoblastoma, and this distinction in the past has relied on anatomic site of origin.

This case highlights the power of genomic analysis to aid in diagnostic evaluation and suggests that *DICER1* alterations and microRNA deregulation may be an important biomarker for pineoblastoma, both in sporadic cases and in patients with *DICER1* tumor predisposition syndrome.

Discussion

Primary neoplasms of the pineal gland are exceedingly rare, and thus little is known about the clinical behavior or biology of these tumors. Here, we present clinical follow-up and histopathologic data from patients with PPT treated at a single institution over the span of 23 years. Our objectives were to define the clinical behavior of PPT according to current histopathologic criteria and identify prognostic factors to guide therapy. Beyond the selection biases that are intrinsic to all retrospective studies, the small sample size here limited our ability to evaluate multiple variables simultaneously and adjust for potential confounders. However, the data from this cohort, which is one of the largest groups of patients with PPT according to current diagnostic criteria, suggest that tumor histology including PPTID subtype, extent of resection, and neuraxis spread are the most important factors for outcome. Moreover, in re-reviewing pathologic specimens from these tumors, we identified 2 novel morphologic subtypes of PPTID that are associated with distinct clinical outcomes.

The 2007 revision of the WHO classification of pineal neoplasms, which included recognition of PPTID as a distinct entity, makes these findings particularly valuable.[3] As expected, the majority of revised cases were initially diagnosed in the prior classification era. Indeed, histopathologic re-review led to the identification of 9 additional cases of PPTID en route to adjusting approximately 25% of the diagnoses. The discovery of so many PPTIDs in older individuals, which were previously reported as pineoblastoma, is most likely indicative of a distinct entity among PPTs and may have significant implications. Consistently, pineoblastoma outcomes from the prior diagnostic era showed more favorable results in older patients, and it is likely that many of these cases would have been reported as PPTID by current standards.[7] Given the comparatively younger age of onset for true pineoblastomas, it is challenging to definitively establish age as an independent prognostic factor for PPT in general, as some have suggested.[8,29] More likely, the relatively superior performance of older patients with PPT is a function of the lower incidence of aggressive neoplasms in this subgroup.

The small-cell and large-cell subtypes of PPTID likely represent 2 genetically or epigenetically distinct PPTs. We anticipate that our ongoing genomic analyses of PPT in this patient cohort will determine the genetic alterations that drive these tumors and may serve as helpful diagnostic and prognostic biomarkers. However, there are no molecular markers known to date that can reliably distinguish between the small-cell and large-cell subtypes. Although sensitive molecular markers of PPT have been identified in several small case series, the generalizability of these markers across PPT histologies is unclear.[35–40] Furthermore, it is unknown whether PPTs exist on a continuum; although there have been 2 reports of low-grade pineal neoplasms transforming to pineoblastomas and one case of pineocytoma disseminating to the leptomeninges, it remains to be established if these are indicative of true tumor biology.[41–43] The evolving understanding of pathological features and modifications in the classification scheme also accounts for some of the discrepancies in the literature. High-resolution genomic analyses will shed light on these ambiguities and also identify molecular markers that may guide chemotherapeutic decisions for future patients. For example, the pineoblastoma case we report with somatic mutation of *DICER1* suggests that *DICER1* inactivation and microRNA deregulation are likely to be important in the pathogenesis of sporadic pineoblastomas, in addition to those arising as part of the *DICER1* tumor predisposition syndrome. The exact frequency of *DICER1* inactivation in pineoblastoma remains to be determined, as does whether *DICER1* and microRNA deregulation help distinguish pineoblastoma from PPTID and pineocytoma. Furthermore, the presence of somatic mutations in the chromatin-remodeling gene *ARID1A* and the histone demethylase gene *KDM5C* suggest that transcriptional deregulation may be another critical pathway in the pathogenesis of pineoblastoma.

Our data support the hypothesis that GTR improves tumor control and survival from pineocytoma.[9,44] Moreover, for the first time we demonstrate that the extent of resection is important for the outcome of other PPT histologies. Radiotherapy techniques were heterogeneous, which was likely a reflection of the 23-year study period. The use of adjuvant radiation for residual pineocytoma is controversial, and it is possible that stereotactic radiosurgery or brachytherapy may yield superior outcomes relative to fractionated approaches.[9,19,21,22,26,44] Nonetheless, disease control and survival are equivalent in PPT patients irrespective of adjuvant therapy, which argues that chemotherapy and radiation are important for patients with high-grade lesions. Indeed, other investigators have reached similar conclusions for the use of stereotactic radiosurgery with residual pineocytoma[4,28] and multimodal therapy for pineoblastoma.[23,33]

Conclusions

Histologic grade, PPTID subtype, extent of resection, and neuraxis spread at diagnosis are important factors for outcome in PPT. Despite the selective application of chemotherapy and radiation in patients with adverse features, individuals who received adjuvant therapy are no more likely to experience disease recurrence or death than those who do not. This suggests that patients with malignant and/or residual grade II or grade III PPT after surgery benefit from adjuvant treatment. However, considering the long survival of many patients with PPT, as well as the adverse late effects of adjuvant therapy, radiation field optimization and use of chemotherapy require prospective investigation to establish optimal treatment regimens, especially for PPTID. In the interim, we recommend intensive multimodal adjuvant therapy for patients with evidence of neuraxis dissemination and prophylactic multimodal adjuvant therapy for all pineoblastomas and select PPTIDs with adverse features such as subtotal resection and, potentially, large-cell morphology.

Supplementary Material

Supplementary material is available at *Neuro-Oncology Journal* online (http://neuro-oncology.oxfordjournals.org/).

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