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Molecular Classification and Management of Rare Pediatric Embryonal Brain Tumors

Patrick Sin-Chan[1] · Bryan K. Li[1,2] · Ben Ho[1] · Adriana Fonseca[1] · Annie Huang[1,2]

Abstract

Purpose of Review Malignant embryonal brain tumors (EBTs) of childhood span a wide clinical spectrum but can share remarkably similar morphologic features. This overlap presents significant diagnostic challenges, particularly for tumor entities that are rarely encountered in clinical practice and for which diagnostic criteria were poorly defined. This review will provide an update on the evolving characterization and treatment of rare EBTs.

Recent Findings Rapid advances in genomic tools have led to the discovery of robust molecular markers, and identification of novel tumor types and subtypes for almost all major categories of pediatric brain tumors. These developments have had significant impact on improving the diagnostic classification of the rare EBTs, particularly for tumors with newly recognized *C19MC* alterations, central nervous system primitive neuroectodermal tumors (CNS-PNET), and pineoblastoma (PB).

Summary These important developments in the clinical and molecular understanding of rare EBTs are paving the way for novel therapeutic strategies and improved clinical management.

Keywords Brain tumor · Cancer · CNS-PNET · PNET · C19MC · ETMR · ETANTR · Ependymoblastoma · Medulloepithelioma · Pineoblastoma · Pediatrics · Therapeutics

Introduction

Brain tumor diagnoses have traditionally been established as per the World Health Organization (WHO) CNS tumor classification criteria based on tumor location and histopathologic features, including tumor grade (I–IV), morphologic features of major CNS cell lineages (glial, astrocytic, neuronal), and more recently the presence or absence of specific molecular alterations [1]. Diagnosis and classification of embryonal tumors, which represent the largest category of brain tumors in children 0–14 years of age [2], have historically been particularly challenging as they often exhibit similar "small round blue cell" histology and were collectively classified as primitive neuroectodermal brain tumors arising in infratentorial (cerebellum/posterior fossa) or supratentorial (cerebral) compartments, and respectively named medulloblastoma (MB) and supratentorial PNET (sPNET). It is now increasingly recognized that various classes of embryonal tumors may arise within infratentorial as well as supratentorial brain compartments, and that tumors with seemingly disparate histopathologic features may represent common molecular diseases. The

Patrick Sin-Chan and Bryan K. Li contributed equally to this work.

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✉ Annie Huang
annie.huang@sickkids.ca

Patrick Sin-Chan
psinchan@gmail.com

Bryan K. Li
bryan.li@sickkids.ca

Ben Ho
ben.ho@sickkids.ca

Adriana Fonseca
adriana.fonseca@sickkids.ca

[1] Arthur and Sonia Labatt Brain Tumour Research Centre, Division of Haematology/Oncology, Hospital for Sick Children, Toronto, Ontario M5G1X8, Canada

[2] Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S3H7, Canada

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notion that embryonal tumors/PNET may comprise a spectrum of molecular entities first came with the identification of atypical teratoid/rhabdoid tumors (AT/RT), based on specific morphology and characteristic *SMARCB1* alterations [3, 4]. The first array-based global gene expression studies consolidated and advanced these observations by demonstrating that childhood MB, AT/RT, sPNET, and high-grade gliomas (HGGs) were transcriptionally distinct [5]. Subsequent discovery of recurrent amplification targeting *C19MC*, a large microRNA (miRNA) cluster, in a subset of sPNET with distinct histology and clinical tempo, led to the recognition of *C19MC*-altered tumors or embryonal tumor with multilayered rosettes (ETMR) as a new and distinct tumor entity [6, 7]. Based on collective global transcriptional and methylation profiling studies [7, 8••], which indicated further molecular heterogeneity in sPNET without *C19MC* alterations, the diagnostic category of sPNET/CNS-PNET has now been revised in the 2016 WHO CNS tumor classification. Pineoblastoma (PB), a distinctive embryonal tumor of the pineal region, which was previously categorized as sPNET in clinical studies, is also now recognized as a separate molecular entity based on global profiling studies, although a recurrent molecular alteration specific to PB remains to be identified.

Historically, all non-MB embryonal tumors including CNS-PNETs/sPNETs and PB were considered more aggressive diseases and collectively treated with intensified high-risk embryonal/MB protocols. The identification of specific molecular entities within this broad umbrella presents new opportunities to better define the natural history and response of specific tumor categories to conventional chemotherapy and radiation-based EBT regimens (Fig. 1). More significantly, these recent developments will advance deeper biological understanding and development of precise disease-specific and biology-tailored therapeutic approaches.

C19MC-Altered Tumors/ETMR

Embryonal tumors with abundant neuropil and true rosettes (ETANTR) were first described as a new histologic category of particularly aggressive, embryonal tumors arising in younger children [9]. Subsequent global molecular studies revealed

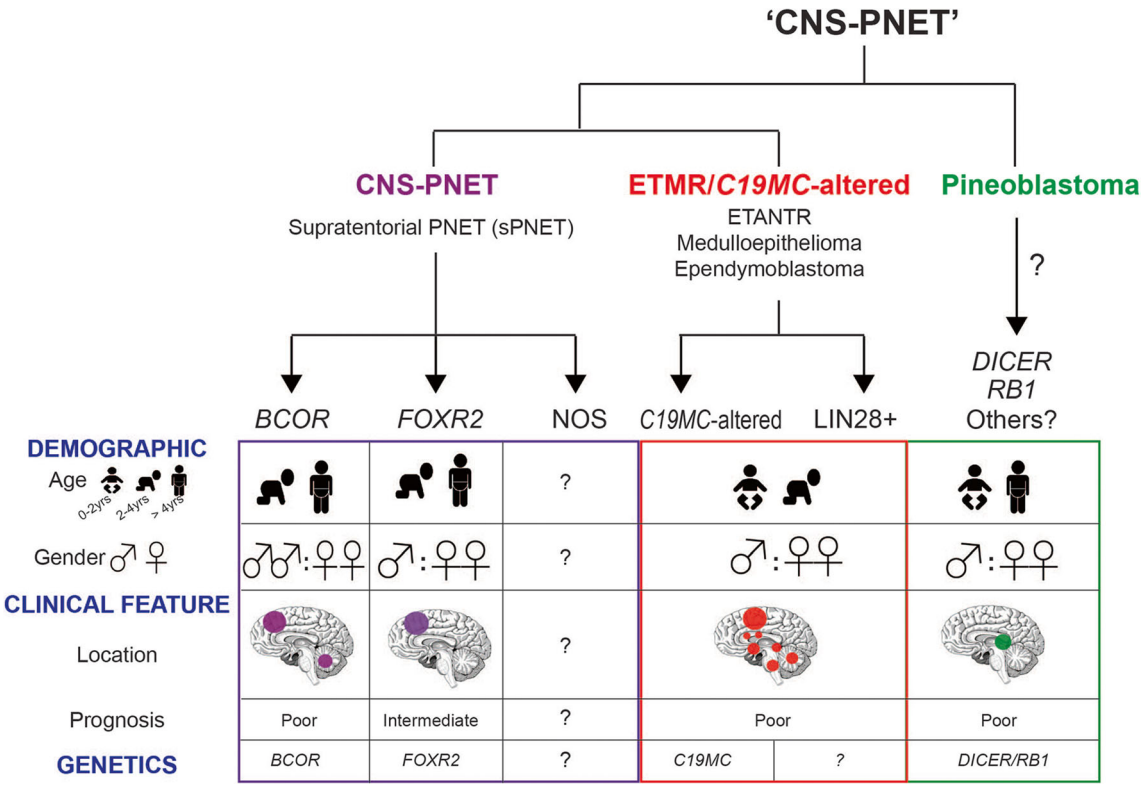

Fig. 1 Summary of recent molecular and clinical classifications of embryonal tumors classified as CNS-PNET, EMTR/*C19MC*-altered, and pineoblastoma. Embryonal tumors, previously grouped under the "CNS-PNET" umbrella, are now known to encompass several distinct embryonic brain tumor entities. CNS-PNET/supratentorial-PNET (sPNET) without *C19MC* alterations are comprised of closely related entities distinguished by genetic alterations of *BCOR* and *FOXR2*, as well as less defined other not-otherwise-specified (NOS) tumors. ETMR/*C19MC*-altered tumors consist of tumors with histologic diagnoses including ETANTR, medulloepithelioma, and ependymoblastoma, which are now known to collectively represent a distinct molecular entity. These tumors may be further subgrouped based on *C19MC* alterations with LIN28 expression (*C19MC*-altered) or LIN28 expression alone (LIN28+). Although the molecular subgroups of pineoblastoma remain unknown, potential subgroups include tumors with genetic alterations of *DICER1*, *RB1*, and others may be predicted. Predominant age and gender demographics, tumor location, prognosis, and genetic drivers are depicted

recurrent amplification of *C19MC*, an oncogenic miRNA cluster on chr19q13.42, in ETANTRs as well as other embryonal tumors classified as sPNETs, ependymoblastoma (EPB), and medulloepithelioma (MEP) [6, 10]. Based on histopathologic analyses, the term ETMR was proposed to reflect this histologic feature found in most, but not all *C19MC*-altered tumors. Transcriptional and methylation studies of large numbers of rare embryonal tumors showed seemingly disparate histologic categories of embryonal tumors with and without *C19MC* amplification comprised a common molecular disease [11••], which led to their categorization under a single diagnostic label in the 2016 WHO CNS tumor classification.

Clinical Features

Approximately ~ 76% of *C19MC*-altered and related tumors arise in the cerebral hemisphere [11••]. With the discovery of the *C19MC*-altered diagnostic marker, they are now increasingly described in other locations including the cerebellum, brainstem, presacral space, and pineal gland [11••, 12], where they can be mistaken for other tumor entities including MB and PB. Brainstem tumors can be radiologically indistinguishable from diffuse intrinsic pontine glioma. Rarely, these tumors can arise as intra-orbital tumors and need to be distinguished from MEP originating in the ciliary bodies [13], which are more benign tumors with distinct molecular features. Although the demographic data of this newly discovered and rare disease remains to be established, studies to date indicate *C19MC*-altered tumors arise predominantly in children < 4 years of age (median age of 2.9 years) and more often in females. Cumulative data suggest ~ 65% of tumors are localized at diagnosis; however, regardless of initial stage, these tumors are consistently reported to have rapid, progressive, treatment-resistant disease [7, 11••].

Histopathology

Molecular studies show that ETMR/*C19MC*-altered tumors encompass histologic entities called ETANTR, MEP, and EPB. They most frequently share features of multilayered and pseudo-stratified rosette structures, appearing as clusters of poorly differentiated or undifferentiated cells with high nucleus to cytoplasm ratio. However, there can be a continuum of morphologic features with ETANTRs exhibiting ependymoblastic rosettes surrounding well-formed central lumen on a background of neuropil, while EPB lacks neuropil and MEP exhibits papillary and tubular structures reminiscent of an embryonic neural tube with variable amounts of neuropil and rosette formation. Furthermore, 20–25% of tumors with molecular features of *C19MC*-altered tumors lack obvious rosette structures or neuropil but exhibit variable differentiation or bland histology [6, 11••, 12], indicating these tumors may exhibit substantial intratumoral cellular heterogeneity.

Molecular and Cytogenetic Characteristics

chr19q13.42 encompasses two embryonic stem cell-enriched miRNAs clusters, *C19MC* and *miR371-373*; however, initial mapping studies showed gene amplification in ETANTRs and related tumors specifically targeted *C19MC*, which encodes 54 miRNAs. However, high levels of *miR371-373*, which is implicated in genitourinary cancers, may be detected in brain tumors with broad chr19q13.42 amplifications that span both miRNA clusters [6]. An initial study of 500 malignant pediatric brain tumors, including MB, AT/RT, ependymoma (EPN), HGG, and choroid plexus carcinomas (CPC) [6, 11••], showed *C19MC* amplifications or copy number gains were restricted to ~ 25% of cerebral embryonal tumors/CNS-PNETs. However, a small proportion of embryonal tumors with no evident *C19MC* alterations share methylation and gene expression signatures with *C19MC*-altered tumors as well as copy number alterations (CNA), the most common being whole chr2 gains [6, 7, 10].

Transcriptional signatures of *C19MC*-altered tumors are enriched for early neural and pluripotency genes including LIN28/LIN28B, suggesting these tumors are highly primitive in nature. Early SNP arrays and FISH studies of a spectrum of pediatric brain tumors showed *C19MC* alterations are specific to this class of EBTs. Although LIN28/LIN28B are highly enriched in *C19MC*-altered tumors, expressions of these genes are not restricted to ETMR/*C19MC*-altered tumors but are also observed in other high-grade tumors including ~ 25% of AT/RTs and ~ 20% of HGGs. While high LIN28 expression supports the possibility of an ETMR diagnosis, LIN28 immunopositivity alone is not sufficient and specific for diagnosing ETMR or related tumors.

Exome sequencing studies of *C19MC*-altered tumors to date have not identified other recurrent alterations, suggesting *C19MC* as the major oncogenic driver in this disease [7, 14•]. *C19MC* is also targeted by recurrent gene fusions to *TTYH1*, a chloride-binding protein with restricted expression in early embryogenesis [14•]. Gene fusions accompany *C19MC* gains/amplification and are detected in some tumors without evidence of *C19MC* CNAs, indicating high *C19MC* miRNA expression can be primarily driven by gene fusion events. Of interest, high levels of *TTYH1* in early embryogenesis are associated with expression of a neural-specific isoform of the de novo DNA methyltransferase, *DNMT3B*, which has led to the proposal that *C19MC* gene fusions entrap cells in a primitive epigenetic state that is prone to neoplastic transformation.

Treatment and Prognosis

As ETMR/*C19MC*-altered tumors have only recently been classified as a distinct entity, there is limited data to guide

prognostication and treatment [15]. Cumulative data suggest *C19MC* patients who received chemotherapy ± radiotherapy had significantly longer survival, compared to untreated patients (median survival of 13 vs. 0.06 months, respectively) [11••]. Complete resection, radiotherapy, and high-dose chemotherapy with autologous stem cell rescue have been associated with higher overall survival [16, 17]. However, despite the application of intensive multimodal therapy, estimated 5-year overall survival (OS) for these young patients is < 10% [7, 11••, 12], underscoring a need for novel agents in this disease.

Preclinical therapeutic studies have been limited by the lack of model system. Studies to date using three available cell lines established from this disease indicate aberrant epigenetic and metabolic signaling is important in tumor cell growth [11••, 18, 19], thus underscoring agents targeting these pathways as candidate therapeutics. Combinations of 5-azacytidine with vorinostat, as well as the insulin-PI3K-mTOR inhibitor, rapamycin, showed synergistic effects on primary ETMR cell line growth [11••, 19, 20]. Similarly, combining differentiation agents like histone deacetylase inhibitors and conventional chemotherapeutics (i.e., gemcitabine and topotecan) may represent attractive options [18, 19]. Recently, therapeutics targeting SHH and Wnt pathways have been proposed as both pathways are upregulated in primary tumors [6, 21], and concurrent activation of these pathways generated tumors with ETMR-like morphology in mice [21].

Future Directions

As with all new diseases, retrospective studies are critical for informing the clinical and therapeutic profile of *C19MC*-altered tumors. Indeed, the formation of a worldwide disease registry and biological repository (http://www.rarebraintumorconsortium.ca) has to date significantly advanced clinical and biological knowledge of this disease. As the rare nature and cellular heterogeneity of this disease lends itself to diagnostic inaccuracies, it will be critical to globally adopt uniform diagnostic methods, which should include FISH or RNA-seq to detect *C19MC* alterations and LIN28 immunohistochemistry (IHC), in routine practice and clinical trials. A significant challenge in advancing therapies for these tumors is the paucity of preclinical in vitro and animal models. These tumors are difficult to propagate in vitro and in vivo, with only three established tumor lines reported. The fidelity of a recently described animal model generated by concurrent WNT activation remains to be fully evaluated, as this model lacks *C19MC* alterations. Generation of *C19MC*-driven models will be important not only for therapeutic studies, but to fully understand the role of this unusual oncogenic locus in this disease.

Other Embryonal Tumors/CNS-PNETs

Prior to the discovery of *C19MC*-altered tumors, all non-MB embryonal tumors were generally considered a single diagnostic group variously labeled as CNS-PNET or sPNET. They are collectively rare diseases estimated to comprise 3–5% of all pediatric brain tumors and generally considered high-risk [1]. Recent global molecular profiling studies have segregated this collective group into additional new molecular categories [1]. International collaborative efforts are underway to gain better understanding of clinical and biological phenotypes associated with these new, rarer tumor categories. Current knowledge is largely based on retrospective treatment data on CNS-PNETs as a single tumor entity. In this section, clinical and therapeutic experience derived from the broad umbrella of CNS-PNETs are described and the most recent specific data available on individual newer entities are included.

Clinical, Imaging, and Histopathologic Features

CNS-PNETs were traditionally considered to arise in extracerebellar sites, with most in younger children with no gender bias [22–24]. Historical reports on "non-pineal PNETs" suggest median patient age at diagnosis of 3.7 years and metastases at diagnosis in ~ 22% of patients. Clinical presentation of CNS-PNETs relates to tumor location, with signs and symptoms of increased intracranial pressure being the most common. Patients may also present with seizures and impaired movement. Imaging features of CNS-PNETs are non-specific and may be difficult to distinguish from those of other malignant cerebral tumors.

Historically, CNS-PNETs were described as tumors with predominant primitive neuroectodermal histology and variable amounts of neuronal, astrocytic, or ependymal differentiation. Histopathologic diagnosis was based on exclusion of tumors with better defined histologic features such as EPN or HGG, thus potentially introducing substantial variation in criteria for diagnosis [25]. The discovery of recurrent histone mutations in malignant gliomas arising in various locations including the cerebrum underscores the challenge of diagnosing CNS-PNETs and conversely some malignant gliomas based on morphologic features alone. It is now increasingly clear that tumors previously called CNS-PNETs/sPNETs identifiable by specific molecular alterations may arise in different brain compartments, and similar to ETMR/*C19MC*-altered tumors, ongoing analyses of large numbers of molecularly defined tumor categories suggest different molecular categories may also be associated with characteristic histopathologic features.

Molecular and Cytogenetic Studies

Rare tumor incidence and lack of robust diagnostic markers or methods make comprehensive and substantive molecular studies of CNS-PNETs difficult to conduct. Early cytogenetic and molecular studies were limited to 10–30 tumors. Nonetheless, these small cohort copy number studies notably showed lack of iso-chromosome 17q and chr22q11.2 loss targeting *SMARCB1/INI1*, which respectively characterize MB and AT/RT [26–28], indicating CNS-PNETs were molecularly distinct embryonal tumors. Copy number studies also suggested substantial tumor genomic heterogeneity with diverse CNAs detected, but most were not recurrent (occurring in < 10–20% of total) [28]. Alterations of chr1p12-22.1, 1q, 8p, 9p, 13q, 14q, and 20, as well as gains/amplifications of *CDK4*, *PDGFB*, and *PDGFRA*, as well as deletions of *CDKN2A/2B* have been reported [7, 26–30]. However, the significance of the alterations reported in these early studies in relation to clinical phenotypes remained unclear [6, 28, 29]. Recent identification of recurrent RB pathway and *PDGFRA/B* genes in cerebral tumors with diagnostic glioma histone gene mutations suggests some of these observations may be due to inclusion of some misdiagnosed malignant gliomas in these early studies.

To date, there have been two large cohort molecular studies of "CNS-PNETs," one conducted by Picard et al., and a more recent study by Strum et al., which used different approaches to delineate the spectrum of primary cerebral PNETs [7, 8••]. After centralized pathologic review of 254 tumors received as CNS-PNETs/sPNET enrolled in the Rare Brain Tumor Registry, Picard et al. eliminated 112 (44%) samples, including those reclassified as AT/RTs, MB, EPN, and malignant glioma using updated histopathologic diagnostic methods (i.e., BAF47 immunostaining to identify AT/RTs). The exclusion of these diagnoses underscores the challenge of characterizing rare tumors archived over a period of significant changes in diagnostic approaches for brain tumors. From the remaining 142 primary hemispheric CNS-PNETs, Picard et al. conducted transcriptional analyses on 51 samples and copy number profiles for 77 samples. They demonstrated three molecular categories of hemispheric CNS-PNETs based on transcriptional analyses which they named groups 1–3 [7]. Group 1 tumors corresponded to *C19MC*-altered tumors with high LIN28 expression [7, 19] which has since been shown by methylation analyses to be a distinct embryonal group of tumors [8••, 11••]. They reported tumors called groups 2 and 3 lacked recurrent, defining CNAs, but were distinguished respectively by enrichment of oligo-neural (*OLIG1/2*, *BCAN*, *SOX8/10*) and mesenchymal differentiation (*COL1A2*, *COL5A*, *FOXJ1*, *MSX1*) genes [7], and specific clinical features including notably a lower incidence of metastases in the oligo-neural group 2 tumors. Given the enrichment of OLIG2 expression, a known glial marker, these observations raised questions regarding whether CNS-PNETs were simply misdiagnosed malignant gliomas.

A more recent study by Strum et al. employed global methylation analyses to examine 323 archived pediatric and adult brain tumors with only an institutional diagnosis of CNS-PNETs/sPNETs and similarly observed that many can be excluded as other diagnoses based on methylation profiles. Based on comparison of methylation profiles to a large number of other adult and pediatric brain tumors and additional RNA-seq analyses on a subset of tumors, they proposed four new CNS tumor entities emerging from the "CNS-PNETs" umbrella: CNS neuroblastoma with *FOXR2* activation (CNS-NB-*FOXR2*), CNS high-grade neuroepithelial tumor (CNS-HGNET) with *BCOR* alteration, CNS-HGNET with *MN1* alteration, and CNS-Ewing's family of tumors with *CIC* gene fusions (CNS-EFT-CIC*)* [8••]. Clinical and molecular features of the CNS-NB-*FOXR2* most closely align with the *OLIG2*-enriched group 2 CNS-PNETs reported by Picard et al. [7, 8••, 31]. Notably, *FOXR2* upregulation is observed in most, but not all, the tumors in this group, though only a subset exhibits *FOXR2* gene fusions indicating molecular heterogeneity and other potential genetic drivers in this tumor category [8••, 32]. Notably, studies by Ho et al. (unpublished) reveal similar *FOXR2* fusions in subsets of high-grade "*MYCN*" gliomas, suggesting *FOXR2* fusions alone may not be adequate for diagnostic identification [32]. The CNS-HGNET-*MN1* group largely comprised tumors with histologic diagnosis of astroblastoma, a more benign-behaving non-embryonal glial tumor [8••]. Gene alterations described in both the CNS-HGNET-*BCOR* and CNS-EFT-*CIC* have been previously reported in extracranial poorly differentiated soft tissue sarcomas [33–35]. Methylation analyses show these intracranial CIC tumors co-cluster with previously characterized CIC soft tissue sarcomas, indicating these represent intracranial, extraparenchymal presentations of the same molecular disease [32, 36]. However, a direct comparison of CNS-HGNET-*BCOR* and extracranial tumors with *BCOR* gene alterations remains pending [8••, 37, 38].

Clinical features of these additional CNS-PNET entities have also been reported, although the number of cases is very limited [8••]. Both *FOXR2* and *BCOR* tumors present in young children < 4 years of age, with a slightly greater female-to-male ratio for *FOXR2* and no gender bias for *BCOR* tumors. Although both tumors primarily arise in the cerebral hemisphere, *BCOR* tumors have also been observed in the cerebellum. In the cohort reported by Sturm et al. consisting of 7 *FOXR2* tumors and 10 *BCOR* tumors with survival data, patients appeared to respectively exhibit intermediate and poor outcomes. Further studies on larger cohorts are needed to better understand the demographics and clinical behavior of these new subgroups.

Treatment and Prognosis

Historically, high-risk MB protocols have been employed for patients with a diagnosis of CNS-PNETs, which would have included *C19MC*-altered tumors and PB. Furthermore, with demonstration that historical cohorts comprise heterogeneous collection of tumors, extraction and interpretation of treatment and outcome data remain challenging. Intervention data will need to be reevaluated with new molecular knowledge. With these limitations, cumulative data indicate "non-pineal CNS-PNETs" have poorer outcomes as compared to MB, with recent estimates of 44 and 39%, progression-free survival (PFS) and OS respectively [39]. Disseminated disease and incomplete surgery appear to be negative prognostic markers with reported 5-year OS of 40–59% and 10–13% respectively for completely vs. incompletely resected tumors by some studies [39–42]. Earlier studies found no prognostic associations for extent of resection, perhaps reflecting evolving methods of tumor diagnosis [43]. While improved survival with craniospinal irradiation (CSI) generally applied in children > 3 years old has been reported, inferior outcomes have been reported with preradiation chemotherapy [22, 44]. A pilot study by Chintagumpala et al. suggests further improvement in 5-year PFS and OS to 78% in older children by combining risk-adapted CSI with tandem cycles of high-dose chemotherapy/stem cell rescue consolidation [45]. Patients classified as high-risk (residual tumor > 1.5 cm^2 or metastatic disease) received 36–39.6 Gy CSI with boost to primary tumor bed to 55.8 Gy or metastatic sites to 50.4 Gy, while average-risk patients received 23.4 Gy CSI with boost to 55.8 Gy to primary tumor bed. Various chemotherapies have been also investigated as potential radiosensitizers [41, 46, 47], including carboplatin in both the phase I/II COG99701 trial which reported 5-year OS and PFS respectively of 44 and 39% for non-pineal sPNET, and ACNS0332, the current phase III high-risk EBT COG trial that enrolled children age > 3 years with high-risk MB, PB, and CNS-PNETs [39]. However, the benefits of carboplatin as a radiosensitizer or isotretinoin in maintenance as prescribed in ACNS0332 remain unclear. Recently, Hwang et al. reported ACNS0332 outcomes for 60 CNS-PNETs characterized using DNA methylation profiling [48]. Similar to the early findings of Picard et al. and Strum et al., 22 of 60 cases enrolled on ACNS0332 were reclassified—most commonly as HGG using contemporary molecular diagnostic methods. As seen previously, reclassified HGG in this study exhibited poor 5-year PFS and OS of 5.6 and 12% respectively. PBs, which were the majority of sPNET/CNS-PNETs enrolled in ACNS0332, had far better outcomes (5-year PFS/OS of 62.8%/78.5%). Notably, no conclusion can be drawn about ACNS0332 efficacy for non-pineal CNS-PNETs as only a few patients with BCOR or FOXR2-altered tumors were enrolled.

As in MB, radiation deferral or avoidance has been often applied for younger patients with pineal or non-pineal PNETs. However, conventional chemotherapy without radiation produced dismal 3-year OS of 17.2% in children < 3 years old [23]. High-dose chemotherapy with autologous stem cell rescue appears to provide some benefits for some of these young patients, with 5-year PFS of 29% for all sPNETs (including pineal region) reported in the CCG-99703 study [49]. Recent findings suggest that AT/RTs and *C19MC*-altered tumors comprise a significant proportion of previously classified CNS-PNETs and PB in younger children < 3 years of age. Thus, similar to studies of CNS PNETs/PB in older children, there are significant limitations to interpreting outcome data based on analyses of archived CNS-PNETs/PB which have not been centrally reviewed using contemporary histopathologic and molecular diagnostic methods.

Future Directions

The discovery of several molecular tumor categories under the previous diagnostic umbrella CNS-PNETs/sPNETs provides new insights and opportunities to further refine diagnosis and treatment of these rare cancers. As large-scale prospective evaluation of the different rare entities poses significant challenges, construction of future trial concepts will be critically reliant on analyses of retrospective patient data. Large-scale studies of CNS-PNETs to date have varied in diagnostic methods used to establish inclusion as CNS-PNETs, and in the age spectrum of included patients. As some of the newly described CNS entities overlap with extracranial peripheral PNET/soft tissue sarcoma entities, a consensus agreement on organization of retrospective data will also be important for defining the clinical profile of truly novel molecular categories of disease, and to exploit some of the therapeutic successes of already well-studied diseases such as CNS sarcomas for which effective treatment paradigms exist. The use of histopathologic terminology, such as CNS-NB to name novel categories of disease with limited to no molecular or clinical overlap with NB, a generally systemic disease, will also need to be addressed to allow uniformity in development of patient workup and treatment concepts.

Validation of some of the novel oncogenes in the appropriate cellular and animal models will be important to facilitate preclinical studies and identification of potential novel therapeutic targets. Recently, a zebrafish model of oligo-neural/NB-*FOXR2* (*OLIG2+/SOX10+*) CNS-PNETs was developed, which helped to identify established MEK inhibitor AZD6244 (selumetinib) as a potential drug for this group of tumors [31]. Notably, as *FOXR2* alterations in other tumors have been described, practical insights may be gained in the

management of historically distinct entities that share common molecular drivers.

Pineoblastoma

A variety of benign and malignant tumors can present in the pineal region. These include pineocytoma, pineal parenchymal tumor of intermediate differentiation (PPTID), papillary tumor of the pineal region, and the most malignant: pineoblastoma (PB) [50]. PBs are aggressive malignant embryonal tumors that account for approximately ~35% of pineal parenchymal tumors and an estimated 0.001% of all primary CNS neoplasms [50–53]. It has a distinct predilection for children and overall outcomes remain poor, with long-term survival rates between 50 and 60%, with younger patients (< 5 years old) faring much worse (15–40%) [39, 50, 54].

Clinical Features

PBs present at a median age of 4.3 years with bimodal peaks around 3 and 9 years, and a male to female ratio of 0.4–0.7 to 1 [50, 55]. Patients present with symptoms of increased intracranial pressure due to obstructive hydrocephalus and may also exhibit decreased visual acuity and Parinaud's syndrome [56–58]. PBs commonly appear as large, invasive, multilobulated pineal masses with hyperdense, heterogeneous contrast enhancement on CT imaging. They present as hypointense to isointense on T1-weighted MRI, and isointense to slightly hyperintense on T2-weighted MRI, again with heterogeneous contrast enhancement [59–63]. Craniospinal dissemination at diagnosis is observed in 25–41% of patients [50, 52, 64].

Histopathology

Like other historical sPNETs, PBs are highly cellular, small round blue cell tumors composed of dense sheets of poorly differentiated cells with hyperchromatic nuclei and high nucleus to cytoplasm ratio [50]. High mitotic activity and necrosis are commonly observed. Neuroblastic (Homer Wright) rosettes and features of retinoblastic differentiation (Flexner-Wintersteiner rosettes and fleurettes) may be observed in PB. The immunophenotype includes neuronal, glial, and photoreceptor markers. Variable expression of synaptophysin is commonly observed, as well as cytoplasmic expression of neurofilament protein, albeit with rarer frequency. Importantly, PBs retain expression of *SMARCB1/INI1* and lack expression of LIN28, allowing differentiation from AT/RT and ETMR/*C19MC*-altered tumors, respectively [65].

Molecular and Cytogenetic Characteristics

Because of its rarity, molecular studies on PB are scarce and limited to small case series. PB may rarely develop in the setting of tumor predisposition syndromes secondary to germline mutation of *RB1* (in a condition termed trilateral retinoblastoma) or *DICER1* that may drive tumor development [66, 67]. In contrast, limited data is available on the genetic and epigenetic landscape of sporadic cases. Nevertheless, frequent alterations of chromosome 1 and losses of all or part of chromosome 9, 13, 16, and 22 have been reported [29, 51, 68, 69]. No abnormalities of *TP53* or *CDKN1A* have been observed, yet overexpression of genes involved in tumor proliferation (*PRAME*, *CD24*, *POU4F2*, *HOXD13*) has been observed in PB and high-grade PPTID [70–72]. The clinical significance of these alterations remains unknown.

Treatment and Prognosis

PBs have often been grouped together with historical sPNET in clinical trials, complicating PB-specific analyses. The overall management of PB has been based on protocols for other high-risk EBTs. Initial steps often involve acute surgical diversion of CSF to relieve obstructive hydrocephalus, usually by endoscopic third ventriculostomy [73]. This technique also allows the collection of tumor biopsies—a critical step to differentiate PBs from other tumors that may occur in the pineal region (i.e., other lower-grade pineal parenchymal tumors, CNS-PNET, AT/RT, HGG, germ cell tumor) [74]. Due to the lack of specific IHC or molecular markers, and the rarity of pineal region tumors, biopsies should be reviewed by pathologists with expertise in pediatric neurooncology.

Disseminated disease and young age at diagnosis (< 5 years in a meta-analysis encompassing 299 patients) are associated with poorer outcomes [52, 58]. Up-front gross tumor resection (GTR) appears to be associated with improved outcomes but remains difficult to attain due to the deep location of the pineal region and proximity to critical neurovasculature [39, 52, 75, 76]. Tate et al. reported a 5-year OS of 84, 53, and 29% among those who underwent GTR, subtotal resection, or debulking respectively [52]. Similarly, in COG99701, a 5-year PFS of 87.5 vs. 41.7% was observed for patients with localized disease who underwent GTR vs. those who did not [39]. Unfortunately, it is estimated that GTR is obtained in just 30% of cases overall [55]. Post-surgical radiotherapy, consisting of local boost and CSI, is associated with improved survival [22, 42, 64]. Infants, who make up a significant proportion of patients with PB, are unable to receive radiotherapy due to the high-risk of severe neurocognitive impairment. Chemotherapy alone for these patients is grossly ineffective at controlling tumor growth at the primary site or preventing leptomeningeal spread, with all reported patients experiencing

a recurrence within 14 months and dying from disease [77, 78]. However, the later use of high-dose chemotherapy with autologous stem cell rescue appears to provide some survival benefit [79, 80]. Preradiation chemotherapy is associated with worse outcomes apparently due to the delay in irradiation [22]. Although the optimal adjuvant chemotherapy regimen remains unclear, in the phase I/II COG99701 trials, the use of carboplatin during radiotherapy in older children followed by 6 months of cyclophosphamide and vincristine was associated with a promising 5-year OS of 81% [39]. However, the more recent and larger ACNS0332 trial did not find additional benefit for carboplatin or isotretinoin for PBs [48].

Future Directions

The lack of specific molecular markers and largely uncharacterized biology of PB has limited the retrospective analysis of studies and the development of targeted therapies. Indeed, our group's pathological review and molecular analysis of archived PBs suggest a significant number of PB cases are instead cases of AT/RT, *C19MC*-altered tumors, HGG, and germ cell tumors. Importantly, molecular characterization of large cohorts of PB cases is currently being performed by multiple research groups, which may soon yield much needed insights into the oncogenic drivers behind PB.

Conclusion

Childhood EBTs span a wide spectrum of molecular entities. Reflecting their diverse biology, current pan-EBT treatment regimens are highly successful for some but largely ineffective for other tumors. CNS-PNETs, ETMR/*C19MC*-altered tumors, and PBs together present some of the greatest challenges in pediatric neurooncology. The rarity of these EBTs and the general lack of specific molecular markers have complicated the study of these aggressive cancers in large clinical trials. The predilection of these tumors for infants and young children has led clinicians to use intensive consolidative chemotherapy regimens with autologous stem cell rescue to avoid the use of radiotherapy. Although such regimens have proven beneficial, overall outcomes remain poor. While historically grouped as one entity based on histopathology, there is now recognition of at least three distinct clinical and biological diagnoses with further new molecular categories, including CNS-NB-*FOXR2*, which remains to be further defined. Investigations currently underway by our group and others to further characterize the molecular drivers behind these malignancies will be critical to inform the development of novel targeted therapies. Such innovations will be essential to improve patient survival and reduce treatment related-morbidity.

Compliance with Ethical Standards

Conflict of Interest Patrick Sin-Chan was an employee of Regeneron Pharmaceuticals at the time this article was written.

Bryan K. Li declares that he has no conflict of interest.
Ben Ho declares that he has no conflict of interest.
Adriana Fonseca declares that she has no conflict of interest.
Annie Huang declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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• Of importance
•• Of major Importance

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