Embryonal Tumors of the Central Nervous System
An Update

Melissa M. Blessing, DO*, Sanda Alexandrescu, MD

KEYWORDS

• CNS embryonal tumors • Medulloblastoma • Embryonal tumor with multilayered rosettes
• Atypical teratoid rhabdoid tumor • High-grade neuroepithelial tumor

Key points

- Embryonal tumors of the Central Nervous System are a group of genetically heterogeneous WHO grade IV neoplasms.
- The understanding of their molecular biology is rapidly evolving and informs diagnosis, prognosis, and treatment options.
- Integration of the histology and molecular test results is essential for prognostic stratification and precise treatment of embryonal tumors.
- Careful tissue allocation for diagnostic and prognostic testing is essential.
- Embryonal tumors can signal the presence of germline tumor predisposition syndromes; interdisciplinary coordination is needed for assessment.

ABSTRACT

Embryonal tumors of the central nervous system (CNS) are rare, high-grade neoplasms predominantly affecting the pediatric population. Well-defined embryonal tumors include medulloblastoma, atypical teratoid/rhabdoid tumor, embryonal tumor with multilayered rosettes, C19MC-altered and embryonal tumor with multilayered rosettes, not otherwise specified, pineoblastoma, pituitary blastoma, CNS neuroblastoma, and ganglioneuroblastoma. Although their prognosis is nearly uniformly poor, the rapidly evolving understanding of their molecular biology contributes to diagnosis, prognosis, treatment, and clinical trial participation. Knowledge of current tumor stratification and diagnostic techniques will help pathologists guide care and preserve tissue for necessary or desired additional testing.

OVERVIEW

Central nervous system (CNS) embryonal tumors (ETs) include medulloblastoma, atypical teratoid rhabdoid tumor (AT/RT), embryonal tumor with multilayered rosettes (ETMR), pineoblastoma, pituitary blastoma, CNS neuroblastoma, ganglioneuroblastoma, and others, including embryonal tumors, not otherwise specified (NOS). They constitute fewer than 1% of all CNS neoplasms, and are seen most frequently between the ages of 0 to 4 years; they are slightly male-predominant.[1]

CNS ET can occur anywhere in the neuraxis. On MRI studies, they are contrast-enhancing, heterogeneous lesions with restricted diffusion due to their high cellularity. They can have necrotic foci, and may contain cysts. Although

Department of Pathology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA
* Corresponding author.
E-mail address: Melissa.Blessing@Childrens.Harvard.Edu
Twitter: @DrMMB (M.M.B.)

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desmoplastic nodular medulloblastomas have a distinct radiographic appearance, most ETs have overlapping radiological features.[2,3] Many demonstrate leptomeningeal involvement and/or neuroaxis dissemination at the time of diagnosis.

The 2016 World Health Organization (WHO) classification of CNS tumors recommends a diagnosis that integrates the diagnostic (and often prognostic) molecular features when possible.[4] Appropriate molecular testing and standardized reporting are essential for prognosis and treatment, and also for clinical trial qualification and epidemiology.

MEDULLOBLASTOMA

Medulloblastomas (MBs) are posterior fossa ETs that constitute 20% of all childhood brain tumors,[1] and approximately 1% of primary CNS neoplasms in adults.[5,6] Imaging studies demonstrate a cerebellar vermis and/or brainstem, or cerebellar hemispheric mass that may involve the fourth ventricle and have leptomeningeal dissemination (**Table 1**).

MBs are classified into 4 histologic and 4 molecular groups. Histologically, MBs are subgrouped into classic, desmoplastic nodular, extensive nodularity, and anaplastic large cell morphology. All are diffusely positive for synaptophysin and can display scattered glial fibrillary acidic protein (GFAP)-positive cells. The Ki67 proliferation index is typically high, and INI1 immunopositivity excludes AT/RT. Representative illustrations of MB histology are depicted in **Fig. 1**.

Classic MB is characterized histologically by dense, small round blue cells with abundant mitoses and apoptosis. They may have Homer Wright rosettes, neurocytic differentiation, vague nodular areas, and other patterns. Classic MB is the largest histologic category and is seen in all molecular subgroups.

Desmoplastic nodular MBs have a characteristic appearance consisting of nodules of maturing neuroblasts in a background of abundant neuropil surrounded by a reticulin-rich network of mitotically active embryonal cells. Notably, this leads to a reversed pattern of staining with strong synaptophysin and low Ki67 in the neuropil-rich islands, and the reverse pattern in the surrounding primitive cells. Internodular-predominant GAB1 (cytoplasmic) and YAP1 (cytoplasmic and nuclear) positivity is typical.[7] Most desmoplastic nodular medulloblastomas have Sonic Hedgehog signaling pathway activation.

Medulloblastoma with extensive nodularity (MBEN) is characterized by an exaggerated desmoplastic nodular pattern with diminished intervening areas occupied by primitive neuroblasts. Their immunohistochemical and molecular profile mirrors desmoplastic nodular MBs.

Large cell/anaplastic medulloblastoma is a histologic subgroup characterized by frequent cells with marked atypia, cell-to-cell wrapping, cells 3 times larger than the surrounding ones, and atypical mitoses. Necrosis and confluent areas of apoptotic figures are frequent. This histologic type can have c*MYC* amplification, which, independently, is an indicator of poor prognosis. *cMYC*-amplified MBs present with leptomeningeal spread and/or distant metastasis in approximately 40% of cases.[8]

Two less common histologic variants include medulloblastoma with myogenic differentiation and medulloblastoma with melanocytic differentiation. These 2 variants do not carry independent prognostic implications. However, both *WNT* activation and *MYC* amplification have been reported in MBs with these morphologies.[9,10]

MOLECULAR SUBGROUPS

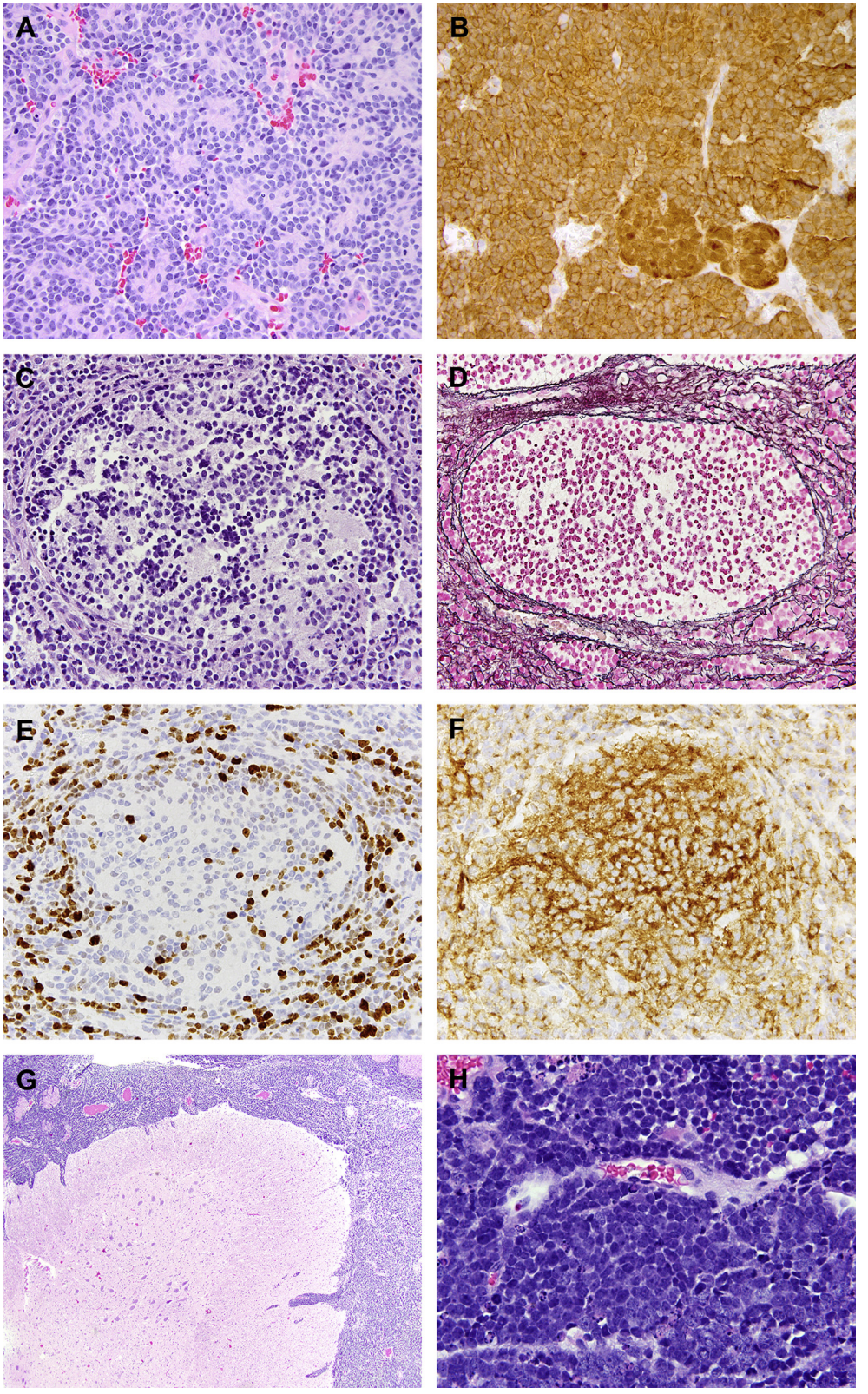
In 2012, an international consensus paper on expression profiling of medulloblastomas showed 4 discrete molecular subgroups with prognostic implications. These include Wnt pathway, Shh pathway, Group 3, and Group 4 medulloblastoma.[11] For practical clinical and prognostic purposes, the 2016 WHO classification of CNS tumors recommends the use of the histologic classification combined with the molecular classification. Because of differences in available modalities and sometimes the necessity for expression or methylation studies to accurately classify molecular groups 3 and 4, the WHO recommends the use of Wnt, Shh with and without *TP53* mutation, and non-Wnt/Shh molecular groups.[4,12,13]

WNT-activated (MB_{WNT}) MBs are the least common (10%) and are seen in older children and adults, and rarely in infants.[4] This molecular subgroup originates from the lower rhombic lip and dorsal brainstem, thus tumors are found along the foramen of Luschka attached to the cerebellar midline or brainstem and extending to the cerebellopontine angle, cisterna magna, or fourth ventricle.[3,14] MB_{WNT} typically has classical morphology. Although beta-catenin is the widest available method to investigate for Wnt pathway activation, its interpretation can be sometimes difficult, as the extent of nuclear translocation varies from diffuse to less than 2% nuclei. Molecular confirmation of *CTNNB1* mutation, typically in exon 3, is recommended. Also, up to 85% of Wnt pathway–activated MBs have monosomy 6,

Table 1
Embryonal tumors of the central nervous system: imaging, histology, and molecular findings

Diagnosis	Location and Imaging Features	Key Histology and Immunohistochemistry	Molecular Alterations and Predisposition Syndromes
MB_{WNT}	Cerebellar midline, along Foramen of Luschka attached to the cerebellum or brainstem; may involve CP angle, cisterna magna, fourth ventricle	Classic histology, no significant LCA Positive: Neuroepithelial markers, INI1 (retained), Beta-catenin (nuclear & cytoplasmic), YAP1 (nuclear & cytoplasmic) Negative: GAB1, H3K27 M	Monosomy 6 *CTNNB1*, *DDX3X*, *TP53*, CSNK2B, *KMT2D, PIK3CA,EPHA7,* SWI/SNF subunits *(SMARCA4,ARID1A* and *ARID2)* In absence of *CTNNB1, APC or AXIN1* mutation (Germline or somatic) Turcot syndrome
MB_{SHH}	Cerebellar hemispheres; sometimes vermis DN/MBEN histology has "grapelike" imaging characteristics *TP53* mutant often have leptomeningeal spread	Wide histologic variety DN/MBEN histology is exclusive to MB_{SHH} *TP53* mutant: Commonly LCA Positive: Neuroepithelial markers, INI1 (retained), YAP1 (nuclear and cytoplasmic) and GAB1 (cytoplasmic) Negative: Beta-catenin (cytoplasmic only), H3K27 M DN/MBEN: Internodular reticulin and high KI67; nodular strong synaptophysin and low KI67.	*TP53* wild-type MB_{SHH}: Chromosome 9q or 10q loss *PTCH1* or *SUFU* (germline or somatic), *SMO*, *GLI2* *DDX3X, KMT2D*; *MYCN, MYCL* amplification Gorlin Syndrome *TP53* mutant MB_{SHH}: Chromosome 17p loss, chromothripsis TP53 point mutations (germline or somatic) GLI, MYCN, SHH amplification Li Fraumeni syndrome HAT complexes, *YAP1*, *BCOR,* rare *IDH1*R132 C, rare germline BRCA2 or PALB2 mutations Adults: PI3K/AKT/mTOR pathway, *TERT* promoter
MB_3	Cerebellar vermis near fourth ventricle	LCA or classic histology Positive: Neuroepithelial markers, INI1 (retained) Negative: Beta-catenin (cytoplasmic only), H3K27 M, YAP1, GAB1	Isodicentric chromosome 17 *MYC*; *MYC* or *MYCN* amplification *GFI1* or *GFI1B* alterations, *OTX2*, *KDM* family, *SMARC4*, *KBTBD4*, *CTDNEP1* and *KMT2D* Notch and TGFβ pathway alterations
MB_4	Cerebellar vermis	Positive: Neuroepithelial markers, INI1 (retained) Negative: Beta-catenin (cytoplasmic only), H3K27 M, YAP1, GAB1	Isodicentric chromosome 17 *MYCN* amplification *SNCAIP* duplication *GFI1* or *GFI1B* alterations, *KDM6A, SNCAIP, CDK6, ZMYM3 or OTX2* Chromatin modification

Abbreviations: CP, cerebellopontine; DN, desmoplastic nodular; HAT, histone acetyltransferase; LoF, loss of function; MB_3, medulloblastoma group 3; MB_4, medulloblastoma group 4; MBEN, medulloblastoma with extensive nodularity; MB_{SHH}, Shh-activated medulloblastoma; MB_{WNT}, Wnt-activated medulloblastoma.



which is not seen in any of the other molecular subgroups. Secondary activation of the Sonic Hedgehog pathway has also been recently described; however, the prognostic significance of such findings is not clear.[15] Other co-occurring genetic events may include mutations in *DDX3X*, *SMARCA4, KMT2D, TP53, KMT2D, PIK3CA*, and *EPHA7*.[4,7,11] In up to 10% to 15% of cases, *APC* mutations activate the Wnt pathway instead of *CTNNB1*; evaluation for Turcot syndrome via germline analysis is indicated in these cases.[16,17]

WNT-activated medulloblastomas with classic morphology have an excellent prognosis in children. They have the lowest probability of metastatic disease, rarely recur, and have a 5-year survival rate of greater than 90%.[7,18] Rarely, large cell/anaplastic (LCA) features are observed; these have an uncertain prognosis.[4,7]

MB, SHH-activated (MB_{SHH}) constitutes approximately 30% of all MBs. MB_{SHH} is thought to originate from cerebellar granule neuron precursors, and involve the cerebellar hemispheres or vermis.[14] They are defined by Sonic Hedgehog pathway activation and further delineated by the presence or absence of *TP53* mutation, which is prognostic in this subgroup. Histone acetyltransferase (HAT), *YAP1*, *TERT*, *PRKAR1A*, and *IDH1*R132 C alterations are also reported.[16,19] MB_{SHH} has the largest variety of morphology. Desmoplastic/nodular medulloblastoma and MBEN variants are most commonly associated with the MB_{SHH} subgroup; both have distinctive histologic and imaging features.[3]

MB_{SHH} wild-type *TP53* has a bimodal age distribution, with most cases occurring in infants and young adults, and an equal sex distribution.[4] MBEN histology is exclusive to this group, and is seen in infants. Germline or somatic *PTCH1* or *SUFU* mutations, or less commonly *SMO* or *GLI2* alterations, activate the SHH pathway. Loss of chromosomes 9q or 10q, *DDX3X* or *KMT2D* mutation, *MYCN* or *MYCL* amplification, *CSNK2B*, *EPHA7*, and SWI/SNF subunit alterations may also be seen.[4,7,16,20] Individuals with Gorlin syndrome, particularly those with *SUFU* mutations, have a high risk of developing MB at a young age.[17,21]

TP53-wild-type tumors with desmoplastic nodular or MBEN histology are low-risk tumors, particularly in infants; classic morphology is standard risk, and LCAs have uncertain prognosis.[4]

MB_{SHH} with *TP53* mutation is rare, and affects older children and teenagers.[22] In addition to *TP53* mutations, MB_{SHH} can have *GLI*, *MYC*, *MYCN*, or *SHH* amplification, 17p loss, and chromothripsis.[4,23] Germline *TP53* mutation testing, diagnostic of Li Fraumeni syndrome, should be considered.[4]

MB_{SHH} with *TP53* mutation has worse prognosis, as it is unresponsive to therapy.[23] Those with classic and LCA histology are high-risk. Although less frequent than in MB_{SHH} *TP53* wild-type, desmoplastic nodular histology is the most common histology in this subgroup and has an uncertain prognosis.[4]

MB group 3 (MB_3) constitutes approximately 20% of all MBs, but almost half of all cases in infants; they frequently (up to 45%) present with metastatic disease and affect boys more than girls.[11] This group is rare in adults.[20] Their origin is in neural stem cells, and they typically involve the cerebellar vermis near the fourth ventricle.[11,24] A subset of MB_3 has c*MYC* amplification, which portents an unfavorable outcome.[7,16,25] MB_3 has LCA or classic morphology and expresses the same neural markers as other MBs. They are negative for GAB1, YAP1, and nuclear beta-catenin.[7] Additional molecular alterations involve *OTX2*, *KDM* family, *SMARC4*, *KBTBD4*, *CTDNEP1*, and *KMT2D*.[16]

MB_3 has the worst prognosis of all MB groups, because of their high rate of metastatic disease and c*MYC* amplification.

MB group 4 (MB_4) is the largest group, constituting at least 40% of all MBs. They are most common in children and teenagers, and are 3 times more common in boys.[4] They are thought to arise from unipolar brush cells (upper rhombic lip), and are found in the cerebellar vermis.[14,24] MB_4 often exhibits classic morphology, but anaplasia may also be seen. In addition to isodicentric chromosome 17 and *GFI1* or *GFI1B* alterations, MB_4 may have *MYCN* amplification and mutations involving *KDM6A*, *SNCAIP*, *CDK6*, *ZMYM3*, or *OTX2*.[16] In addition, 80% of the girls with MB_4 have loss of a

Fig. 1. Classic MB with small, round blue cells, Homer Wright rosettes, and numerous mitotic figures (*A*; 200x); MB_{WNT} demonstrate nuclear beta-catenin positivity, which may be very focal (*B*; 400x). Desmoplastic nodular MB with maturing neuroblasts and neuropil surrounded by embryonal cells (*C*; 400x) and desmoplasia (*D*; 400x). The nodules demonstrate low Ki67 (*E*; 400x) and strong synaptophysin positivity (*F*; 400x), while the surrounding less-differentiated component has the reverse pattern. MB often present with leptomeningeal spread and extend into parenchyma via perivascular (Virchow-Robin) spaces, as pictured in this cervical spinal cord section (*G*; 20x). LCA features may be patchy, and include notably larger neoplastic cells (bottom) which may also have prominent nuclei and/or cell-wrapping (*H*; 600x).

chromosome X, an alteration that is not encountered in any of the other molecular groups.

The prognosis of MB_4 is favorable with classic morphology, and uncertain in LCA morphology. Like MB_3, some present with metastatic disease. The significance of metastatic disease is less clear in MB_4 but is thought to be the main prognostic factor.[11,13]

Additional proposed MB molecular groups are based on DNA methylation profile studies of large pediatric cohorts. A European cohort performed molecular analysis of more than 400 childhood MBs and grouped them into prognostically significant categories by DNA methylation microarray. MB_{WNT} remained unchanged, MB_{SHH} was separated into infant and childhood categories, and the MB_3 and MB_4 groups were each split into low-risk and high-risk groups.[26]

A large international collaboration evaluated DNA methylation and gene expression together via similarity network fusion of more than 700 MBs and discovered 12 prognostically significant categories. Briefly, MB_{WNT} was split between children with monosomy 6 who had good prognosis, and adults with chromosome 6 diploidy and a worse prognosis. MB_{SHH} consisted of 4 groups divided roughly by age and in which *MYCN* amplification and *TP53* are key prognostic factors; among these, some adults were found to harbor *TERT* promoter mutations and have worse prognosis. MB_3 consisted of 3 groups roughly divided by age; one group had a higher frequency of *GFI1* and *GRI1B* oncogenes, and another was high-risk independent of *MYC* amplification. MB_4 consisted of 3 groups again split by age; groups had *MYCN* or *CDK6* amplification, or *SNCAIP* duplications.[19]

Analyses of non-WNT/non-SHH MBs is ongoing. One recent study combined data of the 3 preceding groups with their own, resulting in 8 distinct subtypes with prognostic significance within Groups 3 and 4, based on methylation profiles.[27] Current research is further elucidating MB biology and behavior using single-cell transcriptome analysis.[28,29] Given the fact that use of methylation is not widely spread in clinical settings and given the available treatment protocols for MB, the current practical approach is to separate MBs based on histology and 4 molecular groups, with an emphasis on investigating *cMYC* amplification and anaplastic histology.

ATYPICAL TERATOID/RHABDOID TUMOR

AT/RTs are rare WHO Grade IV tumors typically encountered in children younger than 2 years old, and constitute approximately 15% of ETs in children $\leq$14 years old.[1] AT/RTs have slight supratentorial predominance but can arise anywhere in the neuraxis, including in the pineal and pituitary glands regions, where they have been described in adults.[30–32] Approximately 25% of AT/RTs present with leptomeningeal involvement.[33,34] Histologically, they can be highly variable. Classically they are composed of sheets of cells with reniform or round eccentric nuclei, prominent nucleoli, and eosinophilic cytoplasm; necrosis, mitoses, and hemorrhage are common. However, they may have epithelial and/or mesenchymal differentiation, or be composed of small round blue cells without readily identifiable rhabdoid features. AT/RTs characteristically express focally immunomarkers of all cell lineages (smooth muscle actin, GFAP, synaptophysin, epithelial membrane antigen), and have loss of INI1, or, very rarely, loss of BRG1 immunoexpression.

At a molecular level, AT/RTs usually have homozygous deletion of *SMARCB1* (encoding for INI1 protein); less frequently, AT/RTs can have a combination of *SMARCB1* loss-of-function mutations and heterozygous deletion, or, very rarely, 2 loss-of-function mutations of *SMARCB1*.[35] Very rare cases with homozygous deletion or biallelic loss-of-function mutations involving *SMARCA4* (encoding the BRG1 protein) have been described.[36] Approximately 30% of individuals with rhabdoid tumors, including AT/RT, have rhabdoid tumor predisposition syndrome (RTPS).[21,35,37] This syndrome is characterized by germline alterations in the SWI/SNF chromatin remodeling complex involving *SMARCB1* (RTPS type 1) or *SMARCA4* (RTPS type 2), predisposing individuals to the development of multiple rhabdoid tumors at a young age following a second somatic alteration.[37] Germline testing is recommended in all patients with AT/RT, as carriers of *SMARCB*1 or *SMARCA4* mutation are at risk of developing rhabdoid tumors in other parts of the body as well.[21] Neurovascular hamartoma has been described as a cutaneous stigmata of rhabdoid tumor predisposition syndrome: Perez-Atayde and colleagues[38] reported 2 cases of infants who presented with congenital polypoid skin lesions. At microscopic examination, these skin lesions were characterized by a dermal proliferation of oval cells that expressed S100 and had loss of INI1, admixed with a disorganized network of vascular channels. These cutaneous lesions did not grow, and their histology appeared to be benign. At further imaging, the children were found to have rhabdoid tumors elsewhere in the body. In a recent case in practice, diagnosis of such a lesion led to incidental discovery of AT/RT and

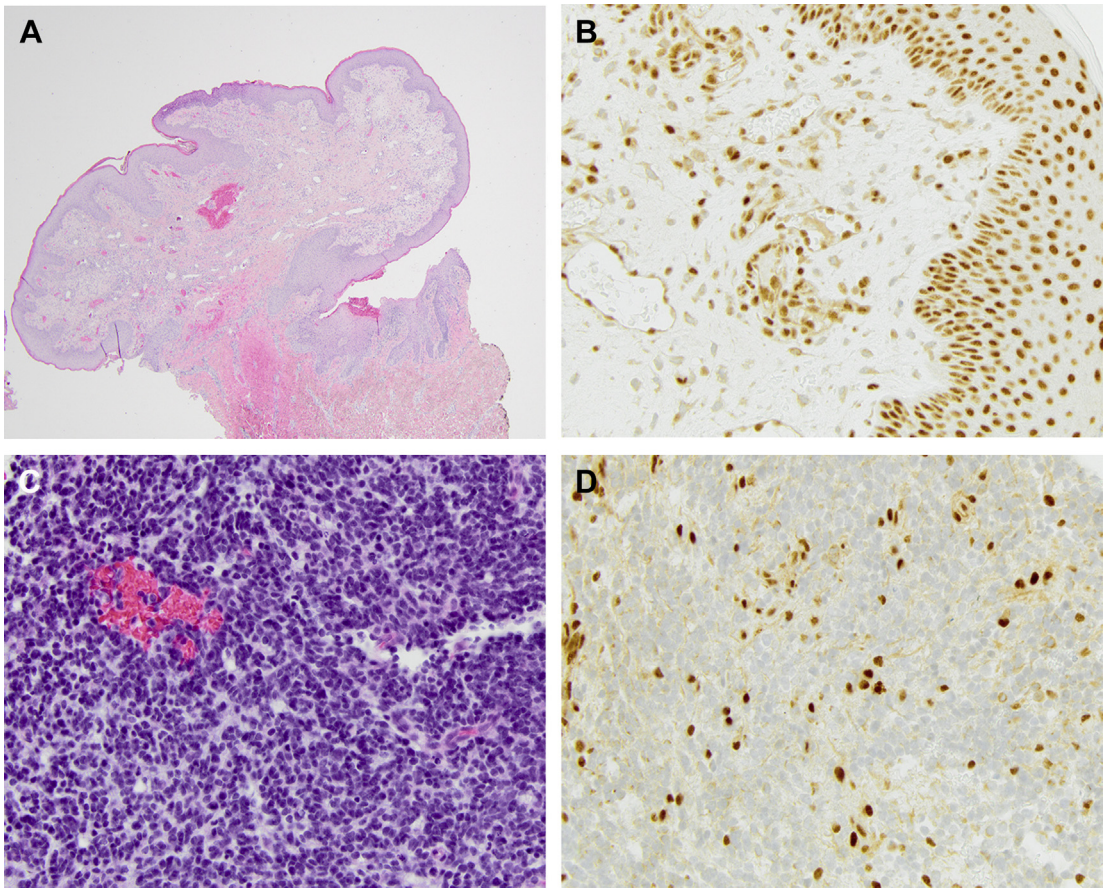


Fig. 2. Neurovascular hamartoma (*A*; 20x) with immunohistochemical loss of INI1 protein (*B*; 400x). ATRT in the same patient, composed of sheets of high grade neoplastic cells including scattered rhabdoid cells (*C*; 400x), and with immunohistochemical INI1 loss (*D*; 400x).

subsequent renal rhabdoid tumor in an infant (**Fig. 2**).

Additional AT/RT groups were proposed following a recent genetic and epigenetic study of pediatric AT/RTs that demonstrated 3 distinct molecular subgroups, summarized in **Table 2**.[39,40] The "ATRT-TYR" group is predominantly infratentorial; these AT/RTs are found in infants, and highly express TYR and other melanosomal markers. The "ATRT-MYC" group is mostly supratentorial, seen in older children, and overexpresses MYC and other HOX proteins. The methylation profile of adult sellar AT/RTs also cluster with this group.[31] The third proposed group, "ATRT-SHH," is seen approximately equally in both compartments, and is defined by

Table 2
Atypical teratoid rhabdoid tumor subgroups

Subgroups	Location	Methylation	Upregulated Pathways, Enhancers, and Enriched Transcription Factors
ATRT-TYR Melanogenesis	Infratentorial > > supratentorial	Hypermethylated	*TYR* or *DCT*, *MITF*, *CCND1*, *VEGFA*, *ERBB2*, ciliogenesis genes, *OTX2*, *LMX1A*
ATRT-MYC	Infratentorial > supratentorial	Hypomethylated	*MYC*, *REST*, *HOX* cluster genes, *ERBB2*
ATRT-SHH SHH pathway	Supratentorial = Infratentorial	Hypermethylated	*MYCN, GLI2, PTCH2, CDK6, FOXK1, ASCL1, HESS5/6, DLL 1/3*

mutations of genes impacting the Shh and NOTCH pathways. The prognosis of these subgroups has not yet been fully elucidated.

There are rare reports of prolonged survival in children and adults with AT/RT,[41,42] but the prognosis is generally dismal. In children, young age and metastasis are independent risk factors for adverse outcome; multimodal treatment may be beneficial in the absence of those risk factors.[41] Adjuvant therapy and additional resection may confer a better prognosis in adults, who are currently treated using standard protocols, the large majority of which were developed based on pediatric studies.[32] The search for therapeutic targets is ongoing.[43,44]

EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES, C19MC-ALTERED AND EMBRYONAL TUMOR WITH MULTILAYERED ROSETTES, NOT OTHERWISE SPECIFIED

The discovery of chromosome 19 microRNA cluster amplification or fusion with *TTYH1* gene in a subset of tumors previously called embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma led to their clustering under the entity ETMR,[45–48] included as an entity in the revised 2016 WHO Classification of Tumors of the Central Nervous System. They are most common in children under the age of 2 years.[4] ETMRs are predominantly seen in the cerebral hemispheres, but can be found anywhere in the neuraxis. They can become very large, extending to involve an entire or both hemispheres, and may be calcified and/or have cystic areas. All have pseudostratified, mitotically active true rosettes with neuroblasts (**Fig. 3**A, B). Three distinct histologic patterns are described as follows.

ETANTR is a biphasic neoplasm composed of primitive small round blue cells arranged in sheets and multilayered true rosettes, admixed with hypocellular areas of neuropil. High mitotic activity, positivity for neural stem cell markers such as nestin, CD99, and synaptophysin, characterize this tumor.[4]

Ependymoblastomas have clusters of multilayered rosettes and embryonal cells, some with fibrillary processes, but lack neuropil and ganglion cells.[4]

Medulloepitheliomas resemble primitive neural tubes. They have tubular, trabecular, and even papillary morphology with distinct, periodic acid-Schiff–positive membranes surrounding these epithelial structures. They lack a prominent neuropil component but may contain mature neurons and astrocytes in addition to embryonal cells, and some display melanin or mesenchymal features.[4] Medulloepithelioma histology warrants a separate, morphologic diagnosis, as the relative paucity of C19MC-alteration in this group suggests a distinct molecular mechanism yet to be elucidated.[4,49]

Aside from the alterations involving C19 MC, these tumors have recurrent copy number alterations of which gain of chromosome 2 is the most frequent; 7q and 11q gains, and 6q loss are also reported. LIN28 A antibody (**Fig. 3**C) is a sensitive immunosurrogate for C19 MC alterations, albeit not specific. The C19 MC alteration can be clinically confirmed by array comparative genetic hybridization. A co-occurring gain of chromosome 2 in this context is also highly suggestive of an ETMR, C19MC-altered.

Recently, Uro-Coste and colleagues[50] (2019) described 2 infants with cerebellar tumors that histologically resembled ETMR and had diffuse LIN28 immunopositivity, but contained heterologous elements (skeletal muscle differentiation in one case and cartilage in the other); both tumors lacked chromosome 19 alteration. On

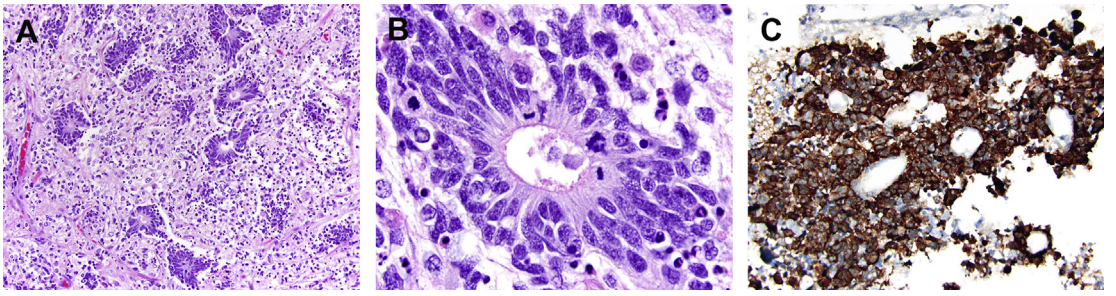


Fig. 3. ETMR, C19MC-altered with ETANTR morphology consisting of a (*A*; 100x) biphasic ET with prominent rosettes, which at higher power are distinguished by pseudostratified, multi-layered rosettes with basally oriented nuclei and numerous mitotic figures surrounding an empty (or amorphous fluid-filled) space (*B*; 400x). LIN28A immunostain is strong and diffusely positive (cytoplasmic) (*C*; 200x).

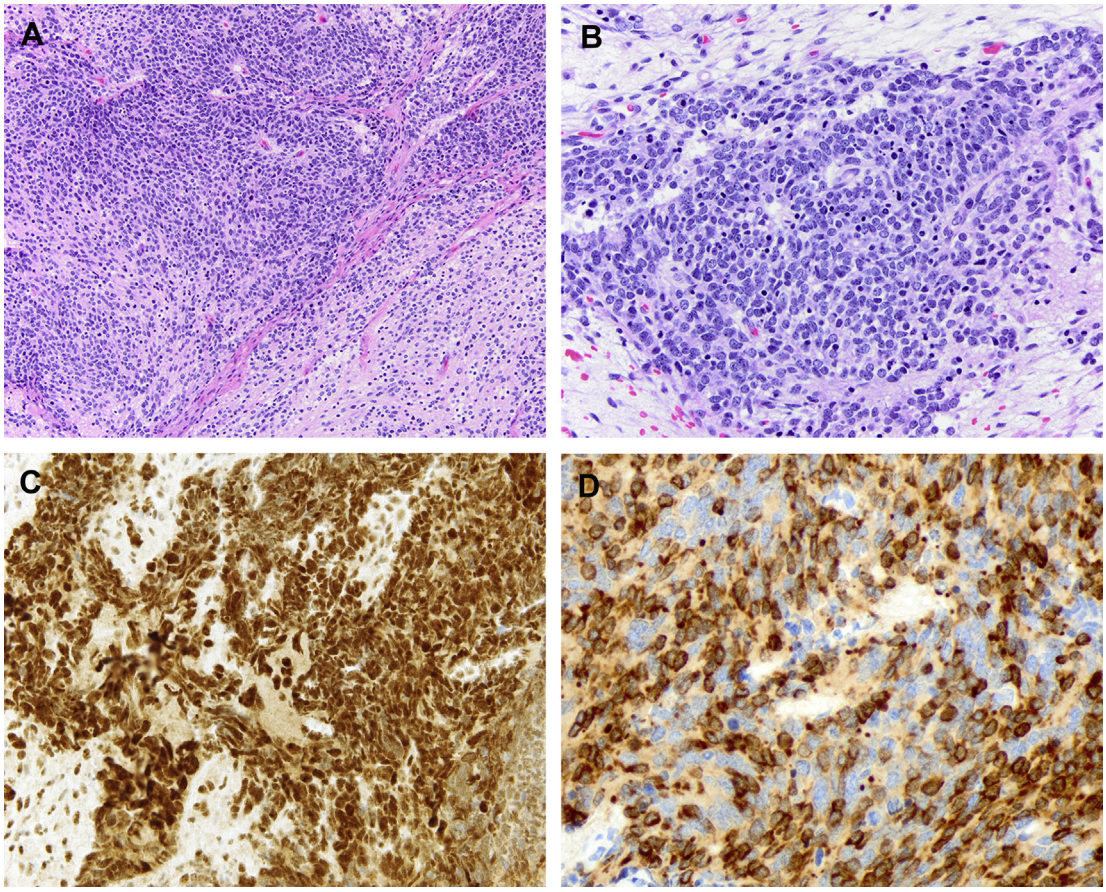


Fig. 4. ETMR-like ET with DICER1 mutation and lacking C19MC alteration composed of (*A*; 40x, *B*; 200x) prominent mutli-layered rosettes and demonstrating immunohistochemical retention of INI1 protein (*C*; 200x) and LIN28 immunopositivity (*D*; 200x).

methylome studies, these 2 tumors clustered separately from, but in close proximity to, ETMRs. Further molecular investigations showed biallelic mutations in the *DICER1* gene, 1 hotspot and 1 missense: a 2-hit mechanism identical to the ones seen in DICER1-associated tumors. An example of such a case is illustrated in **Fig. 4**.

The prognosis of ETMRs is dire. They recur, disseminate, and metastasize. The option of therapy targeting the mammalian target of rapamycin pathway was raised in a cell line study and supported in a subsequent mouse model.[51,52] The mainstay of treatment is still aggressive surgery and chemotherapy; even so, median survival is only a year.[52] Radiotherapy, particularly proton therapy, has recently been shown to prolong survival.[53]

PINEOBLASTOMA

Pineoblastomas (PBs) are poorly demarcated, frequently invasive pineal lesions occurring in the first 2 decades of life. They have typical ET morphology, variable Homer Wright and Flexner-Wintersteiner rosettes, and sometimes heterologous differentiation (**Fig. 5**A). CRX immunoreactivity (**Fig. 5**B), a marker of pineal or retinal origin, distinguishes these from other ETs of the CNS.[54]

The molecular background of PB is not fully elucidated. However, recent studies showed that genes involves in microRNA dysregulation, such as *DICER1* and *DROSHA* play an important role in their genesis, and are mutually exclusive.[55–57] PBs can be seen in the context of *DICER1* tumor predisposition syndrome, where the germline *DICER1* mutation is usually accompanied by loss of heterozygosity of the other allele. A smaller subset of DICER1-associated PBs has biallelic mutations in *DICER1*.[58] If available, DICER1 immunostain will show corresponding loss in the neoplastic cells of tumors with loss of heterozygosity. *DROSHA* is functionally upstream of *DICER1* in the microRNA pathway; homozygous *DROSHA* deletions are also described in PB.[56,58]

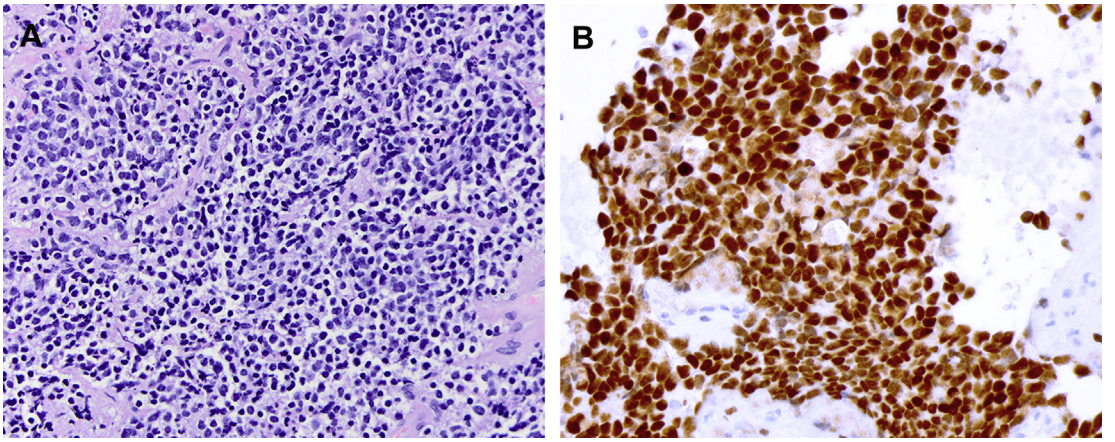


Fig. 5. Pineoblastoma with DICER1 mutation and (*A*; 200) typical ET morphology. CRX immunoreactivity is strong (*B*; 400), distinguishing it from metastatic pleuropulmonary blastoma; the latter is more common in the pediatric population.

In retinoblastoma syndrome, PB is a component of "trilateral retinoblastoma," along with bilateral retinoblastomas.[59]

Like other ETs, PB may present with disseminated disease (approximately 30%); their median survival ranges from 1 to 8 years.[4]

PITUITARY BLASTOMA

Pituitary blastoma is an exceedingly rare embryonal tumor occurring in infants and presenting with Cushing syndrome and diabetes insipidus. Histologically, it is composed of cells that resemble the blastic pituitary gland admixed with Rathke epithelial structures and folliculo-stellate cells.[60] Pituitary blastomas are immunopositive for ACTH and growth hormone, which is a rare combination in pituitary adenomas.

Pituitary blastomas have *DICER1* alterations (14 tested cases presented in literature) similar to those seen in PB, and are characteristic of DICER1 tumor predisposition syndrome.[57,58]

OTHER CENTRAL NERVOUS SYSTEM EMBRYONAL TUMORS

The shift away from histologic categorization and from the PNET bucket diagnostic term was prompted by several important studies showing distinct molecular alteration groupings.[49,61,62] The list of molecularly defined ETs continues to grow, informing diagnosis, prognosis, and treatment.[63–65]

A large international cohort generated DNA methylation profiles of more than 300 "CNS-PNETs" and compared them with a large reference sample. In addition to reclassifying numerous tumors, 4 new entities were discovered: CNS Ewing Sarcoma Family Tumor with *CIC* alteration, CNS High-Grade Neuroepithelial Tumor with *MN1* alteration, CNS High-Grade Neuroepithelial Tumor with *BCOR* alteration, and CNS Neuroblastoma with *FOXR2* (CNS NB-FOXR2) activation.[63] Of those, many have glial or uncertain origin, hence, for the purpose of this review, only CNS NB-FOXR2 activation is discussed.

CNS NB-FOXR2s are ETs morphologically similar to what is currently classified as CNS neuroblastoma (see the following). They have small cell morphology and express OLIG2 and synaptophysin; many contain neuropil and neurocytic or ganglion cells, Homer Wright rosettes, vascular pseudorosettes, and high mitotic activity. In addition to *FOXR2* alterations, chromosome 1q gain and 16q loss are seen.[63]

CNS neuroblastoma and *CNS ganglioneuroblastoma* are classified by the 2016 WHO as unique entities; both are WHO Grade IV. Both typically demonstrate necrosis with granular calcification and palisading and/or Homer Wright rosettes. CNS neuroblastoma exhibit neuropil and neurocytic cells that express neural markers, admixed with primitive cells, whereas CNS ganglioneuroblastomas have neurocytic cells and often-binucleate ganglion cells, and sheets of primitive cells.[4] The prognosis of these exceedingly rare tumors is not certain.

PRACTICE POINTS

At present time, CNS ETs are largely defined by their molecular alterations. Although immunosurrogates for protein products of mutations and

other alterations are more widely used, most are not entirely specific. Therefore, molecular confirmation through DNA-based targeted exome sequencing, array comparative genomic hybridization (C19 MC amplification, for example) or fusion panels when needed, is necessary for a specific, reliable diagnosis that carries important prognostic and therapeutic implications. Methylation, a method of diagnostic clustering that gives copy number information, too, is popular in Europe, and some institutions in the United States have implemented it, albeit mostly as a research modality; it can be particularly helpful in the subclassification of CNS ETs.[16,19,26,27,31,39,40,46,56,63,66,67]

Aside from tissue utilization for a specific integrative diagnosis, almost each of these patients will eventually be enrolled in clinical trials and research protocols. Most pediatric brain tumor clinical trials require 20 to 30 unstained slides and frozen tissue, if available, for enrollment. Hence, mindfulness regarding tissue preservation is necessary, particularly if the specimens are small. Ways to preserve tissue include cutting unstained slides upfront to avoid facing the block multiple times, splitting tissue among multiple blocks to ensure that numerous sections can be cut, and avoiding immunostains that do not bring the case closer to a specific diagnosis meaningful to the patient's future treatment and prognosis. Knowledge of various molecular panels and if/how the result will impact diagnosis, prognosis, and/or treatment is equally important for conservation of tissue. Last, but possibly most important for patient care, seeking expert consultation and molecular testing at an outside institution (if not readily available) in a timely fashion can lead to more precise medical management and ability to participate in clinical trials.

DISCLOSURE

The authors have nothing to disclose.

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