

# **Embryonal tumors in the WHO CNS 5 classification**

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# Embryonal tumors in the WHO CNS5 classification: A Review

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# Abstract: General changes

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Embryonal tumors are a heterogeneous group of neoplasms mostly defined by recurrent genetic driver events. They were previously divided into 2 major categories:

1: medulloblastoma

2: supratentorial primitive neuroectodermal tumors (PNETs)

However, DNA-methylation and gene-expression profiling showed that many tumors previously labeled as PNET actually carried genetic alterations typical of glial tumors, leading to the removal of the term 'PNET' in the 2016 WHO CNS tumor classification.

# Abstract: General changes

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- In the new WHO CNS5 classification, extensive genomic and epigenetic analyses have led to the identification of new molecular entities and the refinement of previous groupings.
- In medulloblastoma, the importance of molecular characteristics has resulted in the definition of Groups 3 and 4 and the integration of both morphological and molecular subtypes into a single unified section.
- Among other embryonal tumors, two newly recognized entities have been introduced:  
CNS neuroblastoma FOXR2-activated and CNS tumor with BCOR-ITD.
- The ETMR category has also been redefined following the discovery of additional DICER1 alterations.
- In AT/RT, three distinct molecular subgroups are now recognized.

**Table 1: Embryonal Tumors: Comparison between 2016 and fifth WHO classification**

*WHO Classification 2016*

*Medulloblastomas, genetically defined*

Medulloblastoma, WNT-activated

Medulloblastoma, SHH-activated, and TP53-wildtype

Medulloblastoma, SHH-activated, and TP53-mutant

Medulloblastoma, non-WNT/non-SHH

*Medulloblastomas, histologically defined*

Medulloblastoma, classic

Desmoplastic/nodular medulloblastoma

Medulloblastoma with extensive nodularity

Large cell/anaplastic medulloblastoma

*Embryonal tumor with multilayered*

*rosettes C19MC-altered*

*Other CNS embryonal tumors*

Medulloepithelioma

CNS neuroblastoma

*Atypical teratoid/rhabdoid tumor*

*CNS embryonal tumor with rhabdoid features*

*WHO Classification 2021 (CNS5)*

*Medulloblastomas*

Medulloblastoma, WNT-activated

Medulloblastoma, SHH-activated, and TP53-wildtype

Medulloblastoma, SHH-activated, and TP53-mutant

Medulloblastoma, non-WNT/non-SHH

Medulloblastomas, histologically defined

*Other CNS embryonal tumors*

Atypical teratoid/rhabdoid tumor

Cribriform neuroepithelial tumor

Embryonal tumor with multilayered rosettes

CNS neuroblastoma, FOXR2-activated

CNS tumor with BCOR internal tandem duplication (ITD)

CNS embryonal tumor NOS

# Molecular Groups of Embryonal Tumors

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- The new classification retains the four main groups from WHO 2016:  
WNT-activated, SHH-activated, non-WNT/non-SHH (Groups 3 & 4).
- In SHH tumors, TP53 status (mutant vs. wildtype) is critical for clinical and biological behavior.
- DNA methylation profiling has identified 12 molecular subgroups:
  - 4 SHH subgroups
  - 8 non-WNT/non-SHH subgroups (Groups 3 & 4)
- This finer stratification has important biological and clinical implications for prognosis and therapy.

# Molecular Groups

## 1. WNT-activated (Wingless/Int-1)

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prognosis: good

Standard therapy: surgery + chemotherapy

Activation of the WNT/ $\beta$ -catenin pathway (commonly CTNNB1 mutations)

## 2. SHH-activated (Sonic Hedgehog)

prognosis: Intermediate

Prognosis depends strongly on TP53 status

TP53-wild type → good outcome

TP53-mutant → poor prognosis

Standard multimodal therapy

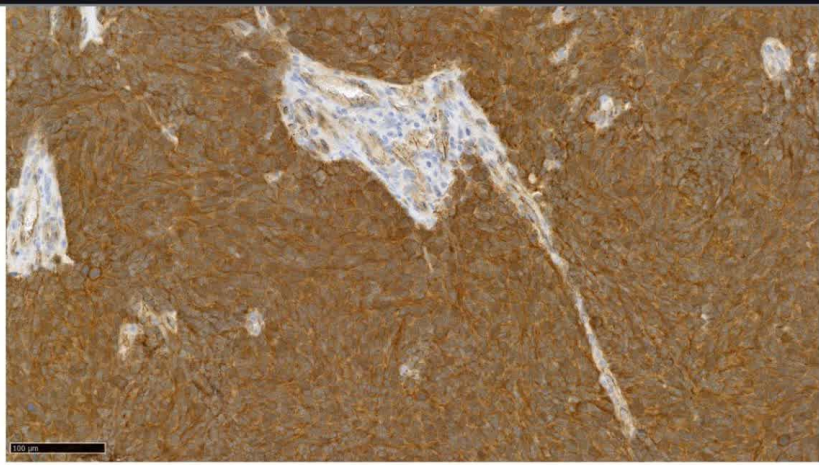
Targeted therapy with SHH pathway inhibitors (e.g., SMO inhibitors) in selected patients

# IHC for group assignment

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Immunohistochemistry can still be used to discriminate between WNT, SHH, and non-WNT/non-SHH medulloblastomas:

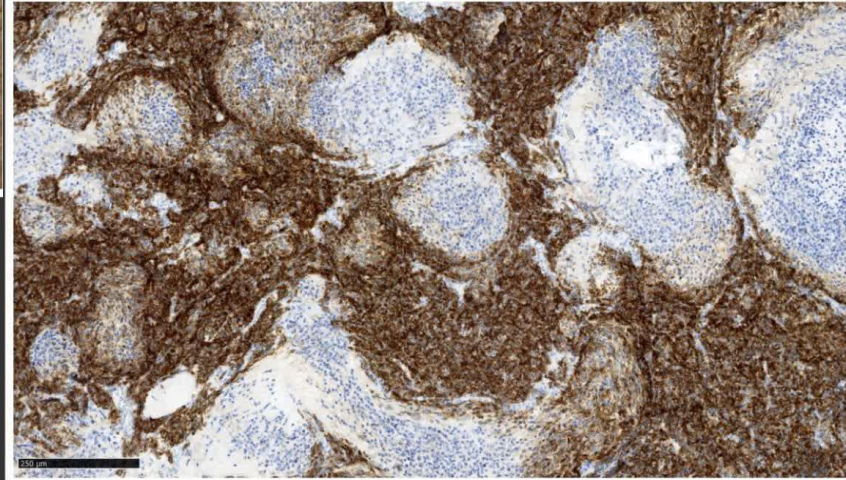
- WNT-activated: Nuclear beta-catenin
- SHH-activated: Cytoplasmic GAB1 and YAP1
- WNT & SHH: Cytoplasmic Filamin A positivity
- Non-WNT/non-SHH: Cytoplasmic beta-catenin, negative for GAB1 and YAP1



Beta catenin immunostaining, magnification 400x, showing a nuclear and cytoplasmic expression in a medulloblastoma, WNT activated. Contributed by Arnault Tauziede-Espariat, M.D.

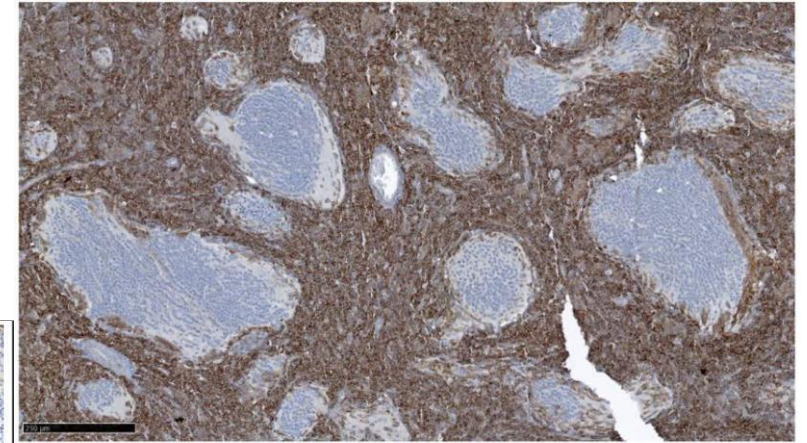
Image 2 of 30

Beta catenin immunoreactivity



YAP1 immunostaining, magnification 400x, showing an internodular expression in a medulloblastoma, SHH activated. Contributed by Arnault Tauziede-Espariat, M.D.

Image 11 of 30



GAB1 immunostaining, magnification 400x, showing an internodular expression in a medulloblastoma, SHH activated. Contributed by Arnault Tauziede-Espariat, M.D.

Image 12 of 30



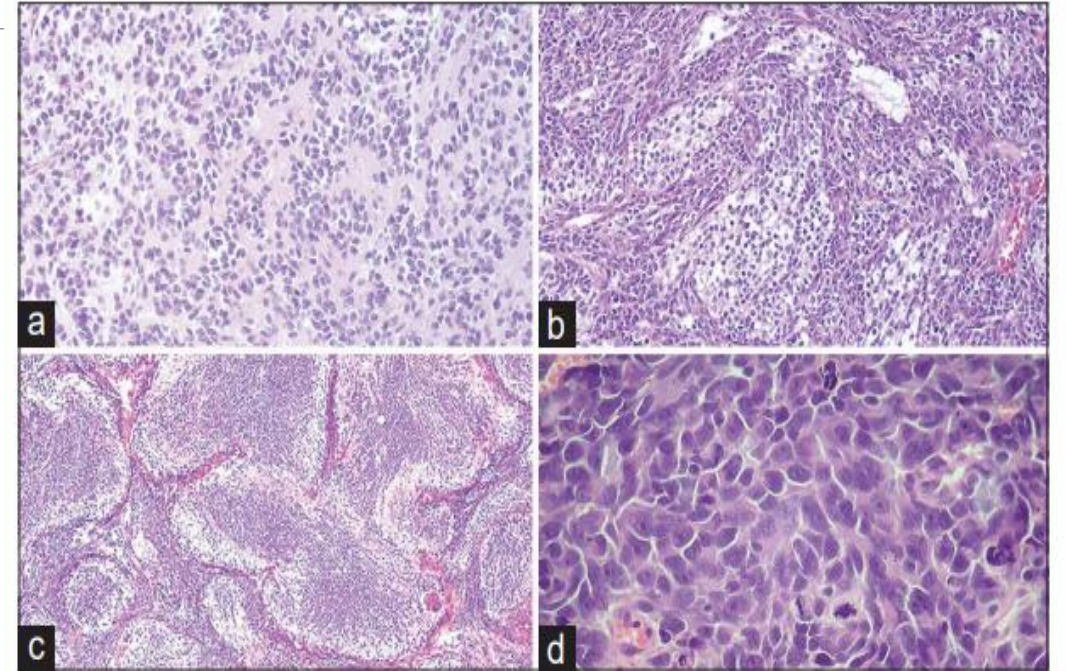
# Histology

WHO CNS5 retains the four histological types from WHO 2016:

- Classic
- Desmoplastic/Nodular
- Medulloblastoma with Extensive Nodularity
- Large Cell/Anaplastic

These are now combined into a single chapter:  
“Medulloblastoma, histologically defined”

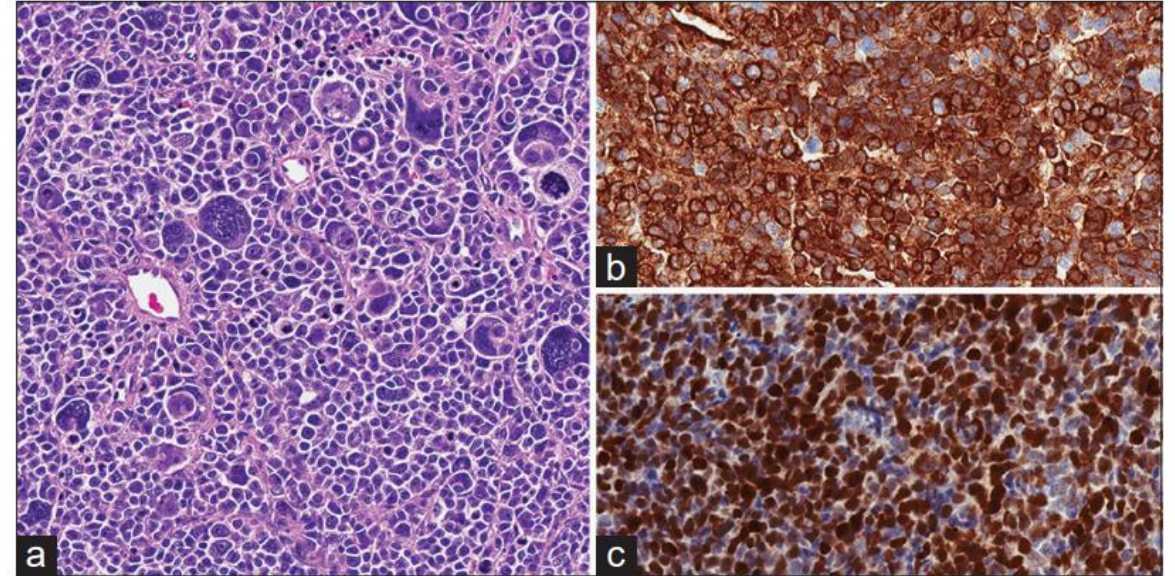
Morphological variation is considered as patterns of a single tumor type



**Figure 2: Different histological types of medulloblastoma (H&E). (a) Classic type (20 ×). (b) Desmoplastic/nodular medulloblastoma (20X). (c) Medulloblastoma with extensive nodularity (10X). (d) Largecell/anaplastic medulloblastoma (40 ×)**

# Molecular subgroup

- Desmoplastic/Nodular & Extensive Nodularity: Mostly SHH, subgroups SHH-1 & SHH-2
- Classic morphology: WNT activated
- Large Cell/Anaplastic: Mostly SHH-3 or non-WNT/non-SHH (Group 3/4) Subgroup 2



**Figure 1: Anaplastic medulloblastoma, SHH subtype, p53 mutated. (a) H and E. Neoplastic cells show some pleomorphic enlarged bizarre nuclei (20 × magnification). (b) Filamin-A immunostain showing a diffuse cytoplasmic positivity (20 × magnification) (c) Diffuse nuclear expression of p53 (20 ×). (By courtesy of Dr. Cynthia Hawkins, SickKids Hospital, Toronto, Canada)**

**Table 2: Clinico-pathological and genetic characteristics of medulloblastoma groups**

Molecular groups	WNT	SHH		G3	G4
		TP53 wild type	TP53 mutated		
Age	Childhood	Infancy/Adulthood	Childhood	Childhood	All age groups
Location	Central, frequently contiguous to brainstem		Hemispheric (rarely midline)	Midline (filling 4 <sup>th</sup> ventricle)	Midline (filling 4 <sup>th</sup> ventricle)
Histology	Mostly classic, rarely large cell anaplastic	Desmoplastic/Large cell/anaplastic	Nodular	Classic, Large cell anaplastic	Classic, Large cell anaplastic
Immunohistochemistry	Nuclear beta-catenin Filamin A positive YAP1 positive GAB1 negative	P53 negative	Cytoplasmic beta catenin Filamin A positive YAP1 positive GAB1 positive p53 positive	Cytoplasmic beta catenin Filamin A negative YAP1 negative GAB1 negative	
Subgroups	$\alpha$ , $\beta$		$\alpha$ , $\beta$ , $\gamma$ , $\delta$	II, III, IV (Group 3)	I, V, VII (Group 3/4)
Genetics	CTNNB1, DDX3X, SMARCA4 and TP53 mutations	PTCH1, SMO, SUFU, TP53 mutation	TERT promoter mutations	MYC, OTX2, SMARCA4, NOTCH, TGF- $\beta$ mutations	VI, VIII (Group 4) MYCN, KDM6A, CDKNA, mutation, SNCAIP duplications
Chromosomal abnormalities	Monosomy of chromosome 6	9q deletion, 10q loss	MYCN amplification, GLI2 amplification, 17p loss	MYC amplification, isodicentric 17q, 1q gain, 5q and 10q loss	MYC amplification, isodicentric 17q, 8, 10 and 11 loss, 4, 7 17, and 18 gain
Outcome of subgroups (5 years survival)	97% ( $\alpha$ ), 100% ( $\beta$ )	69.8% ( $\alpha$ ), 67.3% ( $\beta$ ), 88% ( $\gamma$ ), 88.5% ( $\delta$ )		50% (II) 43% (III) 80% (IV)	77% (I) 59% (V) 85% (VII)
Metastasis (%)	12%	20%( $\alpha$ ), 33%( $\beta$ ), 9% ( $\gamma$ ), 9% ( $\delta$ )		57% (II) 56% (III) 58% (IV)	81% (VI) 81% (VIII) 35% (I) 62% (V) 45% (VII)

# Other CNS Embryonal Tumors

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1. Atypical teratoid/rhabdoid tumor (AT/RT)
2. Embryonal tumor with multilayered rosettes (ETMR)
3. CNS neuroblastoma with FOXR2 activation
4. CNS tumor with BCOR internal tandem duplication (BCOR ITD)

# Atypical Teratoid/Rhabdoid Tumor (AT/RT)

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- Highly malignant, poorly differentiated cells with focal/diffuse rhabdoid features
- Multilineage differentiation with neuroepithelial, epithelial, and mesenchymal phenotypes
- Genetically defined by biallelic inactivation of SMARCB1 (INI1) or rarely SMARCA4 (BRG1)
- Diagnosis:
  - ✓ Based on histology and immunohistochemistry
  - ✓ Loss of SMARCB1 (INI1) expression (or SMARCA4 in rare cases)
- CNS5 Updates – Molecular Subgroups:
  - AT/RT-TYR
  - AT/RT-SHH
  - AT/RT-MYC

**Table 3: Clinico-pathological and genetic characteristics of AT/RT molecular subgroups**

<i>Molecular subgroups</i>	<i>AT/RT-SHH</i>	<i>AT/RT-TYR</i>	<i>AT/RT-MYC</i>
Incidence	44%	34%	22%
Age	2-5 years (median age: 20 months)	0-1 years (median age: 12 months)	>3 years (median age: 27 months)
Location	More frequently supratentorial	More frequently infratentorial	More frequently supratentorial (rarely spinal)
Copy Number Alteration of Chromosome 22	None	Complete loss (monosomy)/partial loss	None
SMARCB1 alterations	Point mutations/Focal deletions	Point mutations/Focal deletions	Extensive deletions
Involved pathway	SHH and NOTCH pathway	BMP and melanosomal pathway	Overexpression of MYC gene and HOX cluster genes

# AT/RT Molecular Subgroups

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## □ AT/RT-TYR (~34%)

Upregulation of melanosomal pathway (tyrosinase)

Median age: ~12 months, mainly infratentorial

SMARCB1 loss: mutation in one allele + complete/partial loss of chromosome 22

## □ AT/RT-MYC (~22%)

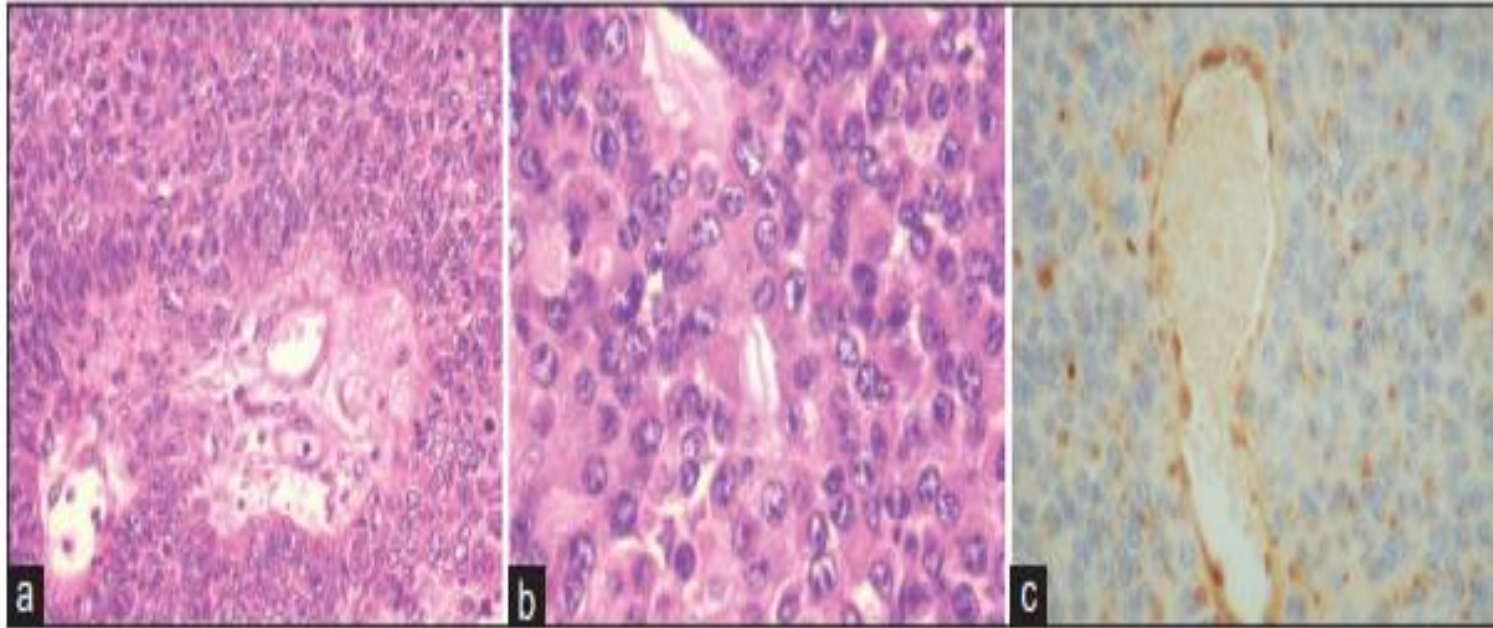
Expression of MYC

Median age: ~27 months, mostly supratentorial, rare spinal cord; adult cases in sellar region

## □ AT/RT-SHH (~44%)

Defined by exclusion from TYR and MYC subgroups

Median age: ~20 months, mainly supratentorial



**Figure 3: ATRT. (a) H and E. Neoplastic rhabdoid cells display a perivascular arrangement (20 x). (b) The tumor has medium-sized, round cells with distinct borders, eccentric nuclei, and prominent nucleoli (H and E) (40 x). (c) INI1 immunostain showing positive endothelial and reactive cells. Tumor cells are negative for INI1 protein (20X)**

# Cribiform Neuroepithelial Tumor

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- A provisional entity distinct from AT/ RT
- A non-rhabdoid neuroectodermal tumor characterized by cribriform strands and ribbons and showing loss of nuclear SMARCB1 expression
- Occurs mostly in periventricular areas (lateral, third, and fourth ventricles)
- IHC staining:
  - Strong EMA positivity
  - Loss of SMARCB1 expression
- Molecular Profile:

DNA methylation clusters within AT/RT-TYR molecular subtype.

# Embryonal Tumor with Multilayered Rosettes (ETMR)

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- ETMR is a unified diagnosis encompassing ependymoblastoma, ETANTR, and medulloepithelioma.
- Characterized in ~90% of cases by C19MC microRNA cluster amplification.
- WHO 2016 named it: “ETMR, C19MC-altered.”

Discovery of frequent biallelic DICER1 mutations in C19MC-negative tumors (often with a germline first hit) led WHO CNS5 to remove “C19MC-altered” from the nomenclature.

ETMRs most commonly arise in the cerebral hemispheres, but may also occur in the cerebellum and brainstem.

- IHC: strongly positive LIN28A (cytoplasmic)

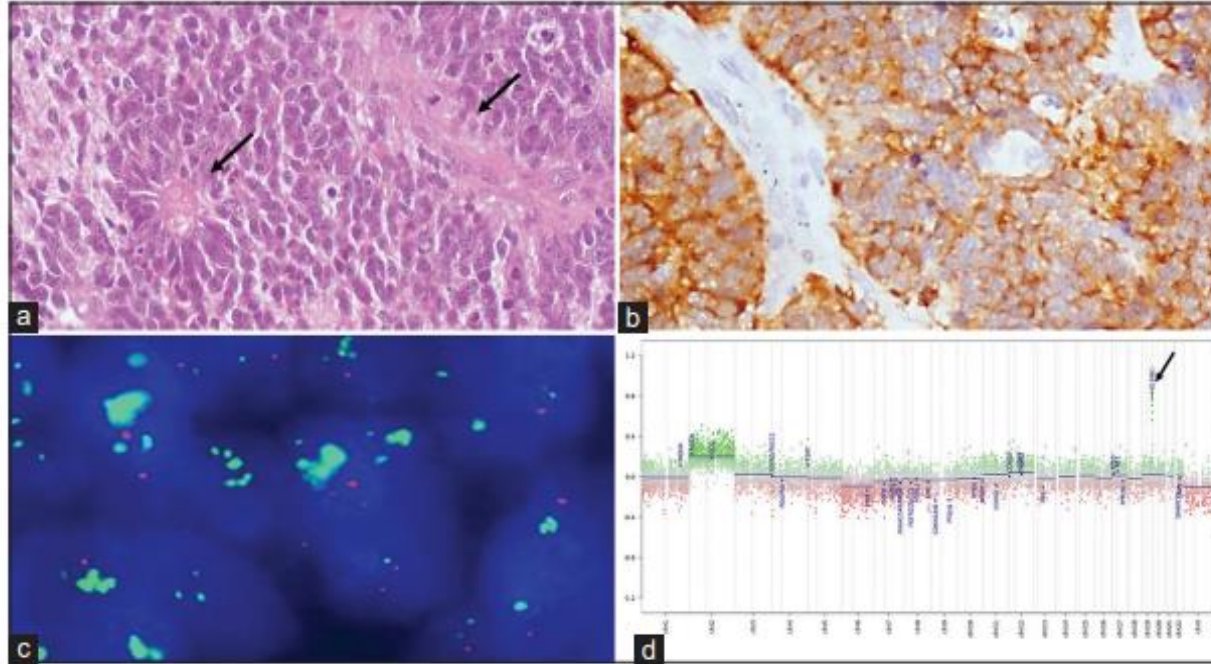


Figure 4: ETMR. (a) H and E. Neoplastic cells with hyperchromatic nuclei. Multilayered rosettes (arrows) are identified. (20x). (b) Immunohistochemistry for LIN28 showing strong cytoplasmic stain (20x). (c) FISH shows amplification at 19q13.42 (green signals). (d) Copy number variation analysis with *C19MC* amplification (arrow)

# FOXR2-Activated CNS Neuroblastoma (CNS NB-FOXR2)

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- Newly recognized entity in WHO CNS5.

Identified through DNA methylation re-evaluation of former CNS-PNET cases.

Characterized by FOXR2 gene overexpression due to chromosomal rearrangements (Xp11.21).

- Histology:

Embryonal architecture with densely packed undifferentiated cells.

Hyperchromatic nuclei, minimal cytoplasm, sheet-like pattern.

Vascular pseudorosettes and Homer-Wright rosettes may be present.

Frequent mitoses, apoptotic bodies, and necrosis.

Focal neurocytic/ganglionic differentiation in some cases.

# FOXR2-Activated CNS Neuroblastoma (CNS NB-FOXR2)

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- Immunoprofile:

Strong OLIG2 positivity.

Negative for GFAP and vimentin.

Areas of neuronal differentiation positive for synaptophysin.

Most cases show TTF-1 overexpression

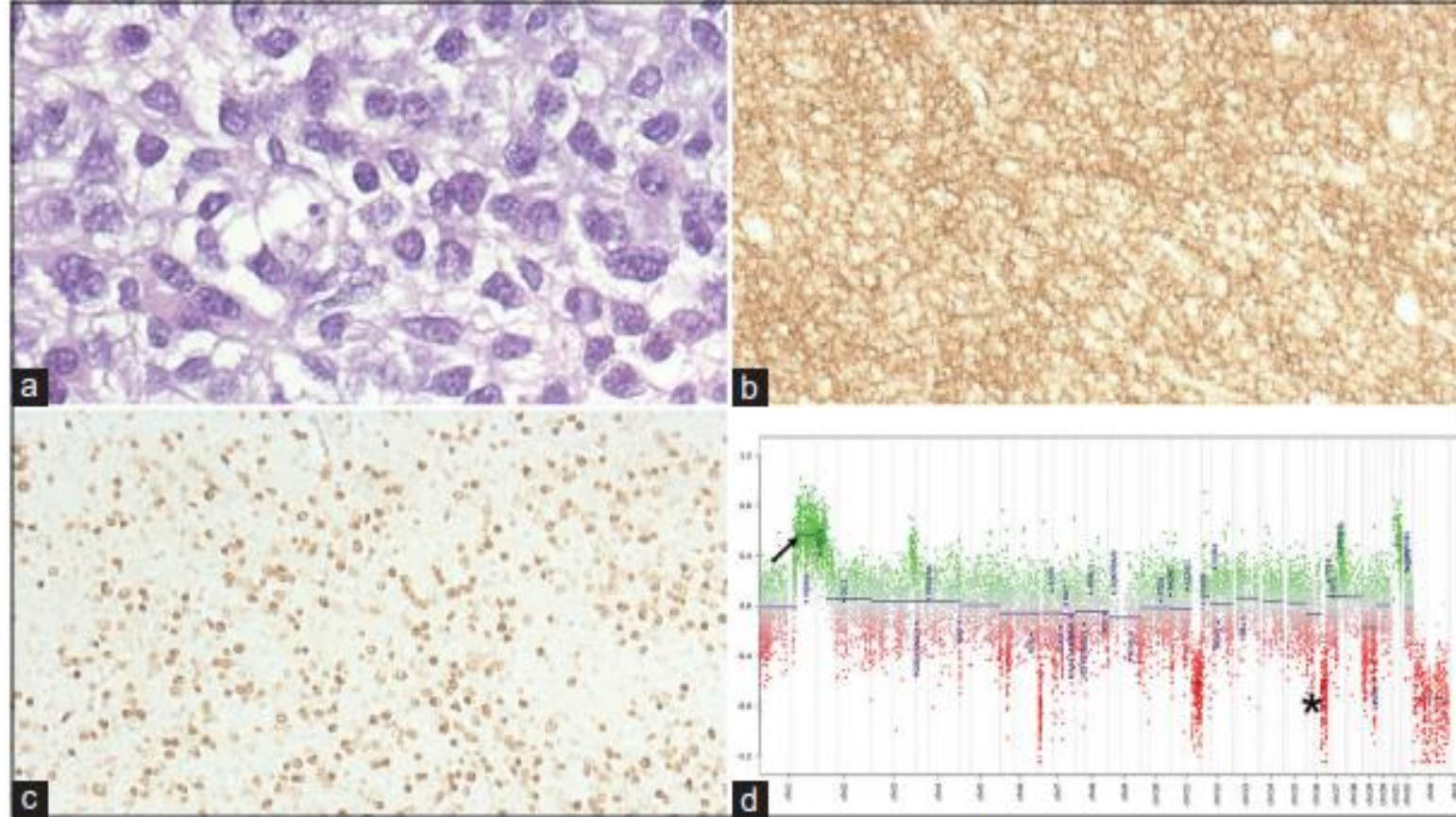


Figure 5: CNS neuroblastoma, FOXR2-activated. (a) H and E. Neoplastic cells with small, round nuclei surrounded by a clear halo (40 x). (b) Synaptophysin immunopositivity (10 x). (c) Olig2 immunopositivity (10 x). (d) Copy number variation. Gain of chromosome 1q (arrow) and focal or total loss of 16q (star)

# CNS Tumor with BCOR Internal Tandem Duplication (BCOR-ITD)

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- Provisionally included in WHO CNS5 as an embryonal tumor; neuroectodermal origin remains uncertain.

BCOR exon 15 ITDs also found in similar sarcomas → unclear if neuroepithelial or mesenchymal.

Occurs mainly in young patients (0–22 years).

Typically located in cerebral or cerebellar hemispheres.

- Histology:

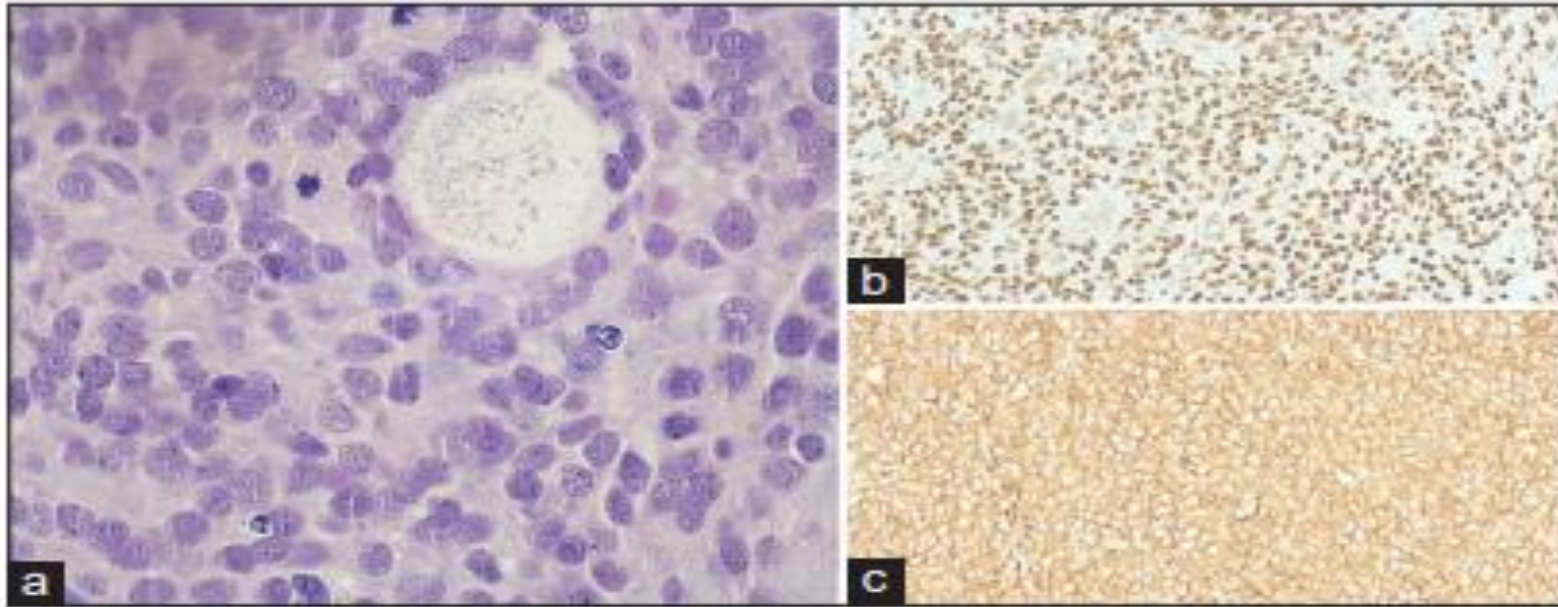
Uniform oval or spindle-shaped cells with delicate chromatin.

Glioma-like fibrillary areas and compact fascicular patterns.

Ependymoma-like perivascular pseudorosettes characteristic.

Myxoid/microcystic areas, frequent mitoses, and palisading necrosis.

- Differential diagnosis: high-grade gliomas, ependymomas



**Figure 6: CNS tumor with BCOR internal tandem duplication (ITD). (a) H and E. Tumor cells show an oligo-like aspect with monotonous round to oval nuclei, fine chromatin, and indistinct nucleoli. Evident some microcystic formation (40 ×). (b) Nuclear BCOR immunostaining (10 ×). (c) Diffuse immunopositivity for vimentin (10 ×)**

# CNS Tumor with BCOR-ITD

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- Positive: vimentin, CD56
- Negative/low: OLIG2, GFAP, S100
- Strong nuclear BCOR expression (sensitive but not specific).
- Definitive diagnosis: detection of heterozygous ITD in BCOR exon 15.
- DNA methylation and gene expression profiling help distinguish BCOR-ITD tumors from other CNS tumors.
- Clinical outcome: limited data due to rarity, but overall survival is generally poor.

# Initial IHC Approach for Non-Medulloblastoma Tumors

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- Start with three simple antibodies: synaptophysin, vimentin, Olig2.

These provide initial clues and guide further testing.

## **CNS NB-FOXR2 suspicion:**

- ✓ Morphologic embryonal tumor with Olig2 + synaptophysin positivity.
- ✓ Next step: molecular confirmation of FOXR2 rearrangement.

## **ETMR suspicion:**

Tumor with undifferentiated small cells + multilayered rosettes.

- ✓ Positive for synaptophysin, Olig2, vimentin.
- ✓ Next step: LIN28 IHC → if positive, confirm C19MC alteration via sequencing or FISH

# BCOR-ITD and AT/RT

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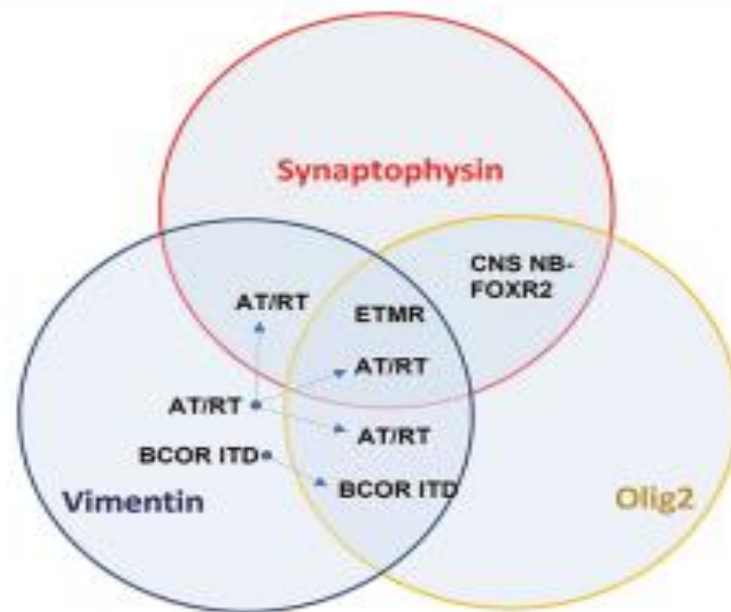
## **BCOR-ITD suspicion:**

- ✓ Poorly differentiated neoplasm, vimentin ± Olig2 positive.
- ✓ BCOR IHC helpful (not specific).
- ✓ Molecular analysis: BCOR exon 15 ITD detection required.

## **AT/RT suspicion:**

Neoplasm with rhabdoid morphology + polyphenotypic differentiation.

- ✓ Vimentin ± synaptophysin + Olig2 positivity.
- ✓ Confirmed by loss of INI1 or BRG1 protein.



- CNS *BCOR* ITD → *BCOR* positive (IHC) \* → *BCOR* ITD detection
- AT/RT → *INI1*, *BRG1* loss (IHC)
- ETMR → *LIN28* positive (IHC) → C19MC detection or *DICER1* mutation
- CNS-NB *FOXR2* → *FOXR2* rearrangement detection

\*not specific of CNS *BCOR* ITD tumors

**Figure 7: Diagnostic algorithm for approaching diagnosis of non-medulloblastoma embryonal tumors, including the integration of immunohistochemical and molecular analysis**



**Thank You**

