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

Embryonal tumors are a heterogenous 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

- 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	*WHO Classification 2021 (CNS5)*
Medulloblastomas, genetically defined	*Medulloblastomas*
Medulloblastoma, WNT-activated	Medulloblastoma, WNT-activated
Medulloblastoma, SHH-activated, and TP53-wildtype	Medulloblastoma, SHH-activated, and TP53-wildtype
Medulloblastoma, SHH-activated, and TP53-mutant	Medulloblastoma, SHH-activated, and TP53-mutant
Medulloblastoma, non-WNT/non-SHH	Medulloblastoma, non-WNT/non-SHH
Medulloblastomas, histologically defined	Medulloblastomas, histologically defined
Medulloblastoma, classic	*Other CNS embryonal tumors*
Desmoplastic/nodular medulloblastoma	Atypical teratoid/rhabdoid tumor
Medulloblastoma with extensive nodularity	Cribriform neuroepithelial tumor
Large cell/anaplastic medulloblastoma	Embryonal tumor with multilayered rosettes
Embryonal tumor with multilayered	CNS neuroblastoma, FOXR2-activated
rosettes C19MC-altered	CNS tumor with BCOR internal tandem duplication (ITD)
Other CNS embryonal tumors	CNS embryonal tumor NOS
Medulloepithelioma	
CNS neuroblastoma	
Atypical teratoid/rhabdoid tumor	
CNS embryonal tumor with rhabdoid features	

Molecular Groups of Embryonal Tumors

- 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/β-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

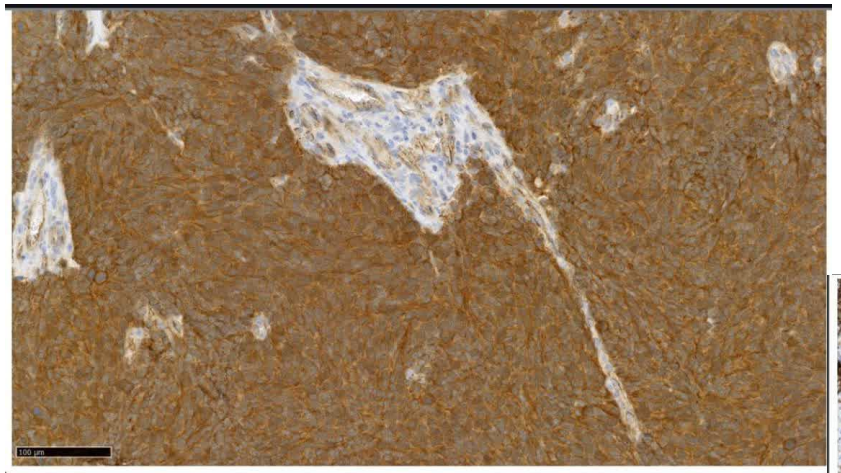
Standard multimodal therapy

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

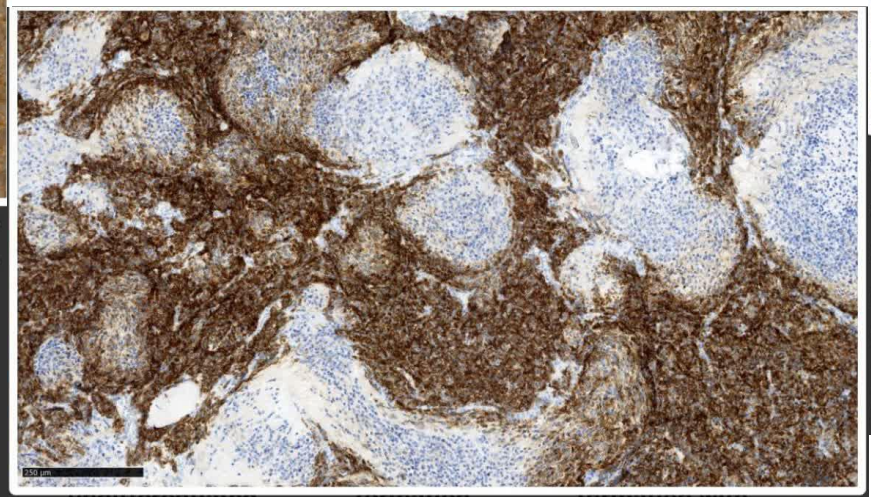
IHC for group assignment

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

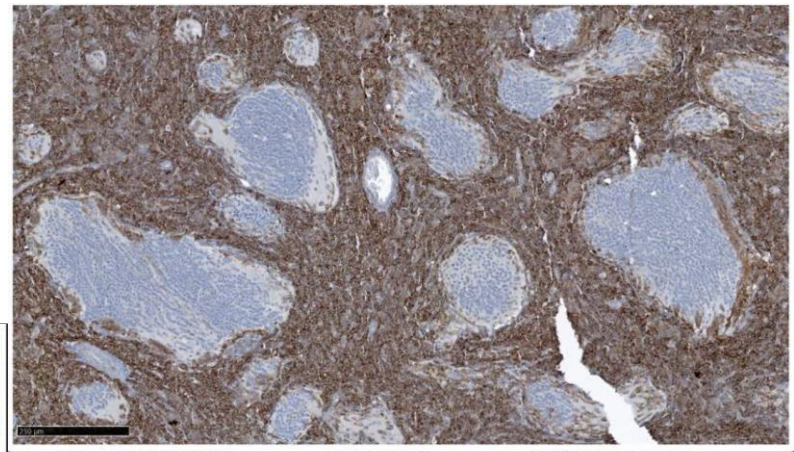
- WNT-activated: Nuclear beta-catenin
- SHH-activated: Cytoplasmic GAB1 and YAP1
- WNT & SHH: Cytoplasmic Filamin A positivity
- Non-WNT/non-SHH: Cytoplasmic beta-catenin, negative for GAB1 and YAP1



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



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



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

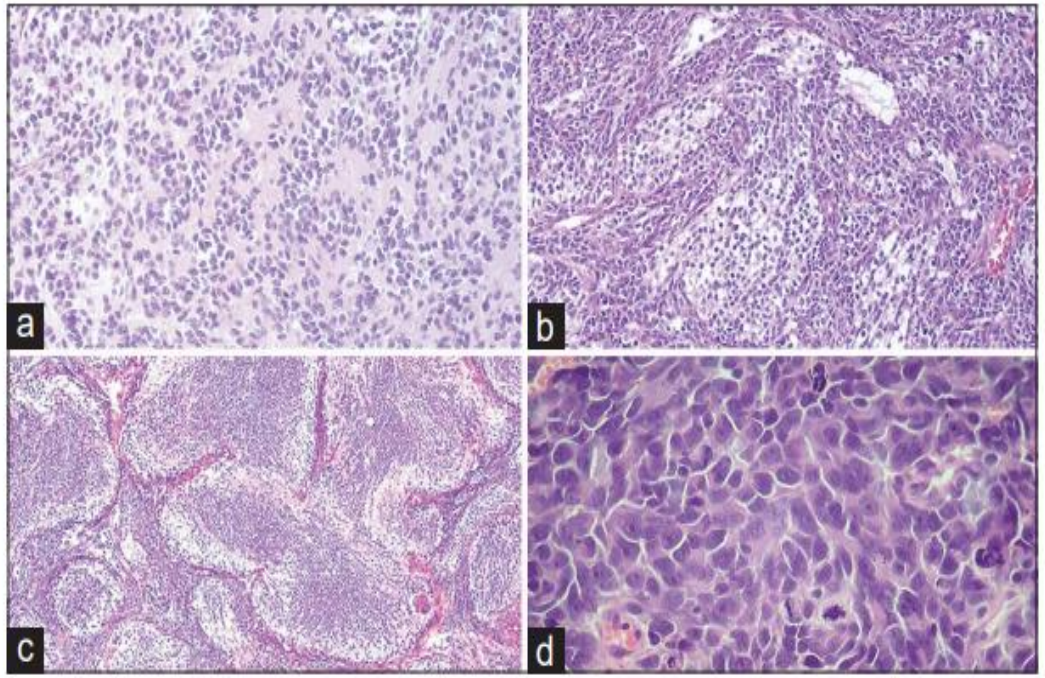

Figure 2: Different histological types of medulloblastoma (H&E). (a) Classic type (20 ×). (b) Desmoplastic/nodular medulloblastoma (20X). (c) Medulloblastoma with extensive nodularity (10X). (d) Largecell/ anaplastic medulloblastoma (40 ×)

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

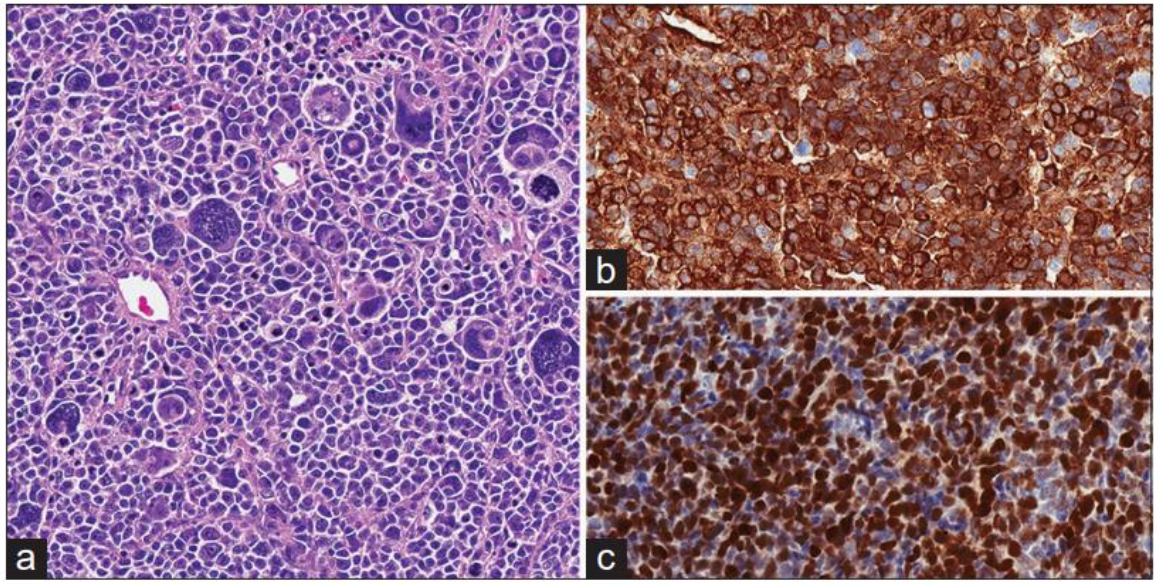

Figure 1: Anaplastic medulloblastoma, SHH subtype, p53 mutated. (a) H and E. Neoplastic cells show some pleomorphic enlarged bizarre nuclei (20 × magnification). (b) Filamin-A immunostain showing a diffuse cytoplasmic positivity (20 × magnification) (c) Diffuse nuclear expression of p53 (20 ×). (By courtesy 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	Childood	Infancy/Adulthood	Childood	Childood		All age groups	
Location	Central, frequently contiguous to brainstem		Hemispheric (rarely midline)	Midline (filling 4th ventricle)		Midline (filling 4th ventricle)	
Histology	Mostly classic, rarely large cell anaplastic	Desmoplastic/Large cell/anaplastic	Nodular	Classic, Large cell anaplastic		Classic, Large cell anaplastic	
Immunohisto chemestry	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	α, β		α, β, γ, δ	II, III, IV (Group 3)	I, V, VII (Group 3/4)		VI, VIII (Group 4)
Genetics	CTNNB1, DDX3X, SMARCA4 and TP53 mutations	PTCH1, SMO, SUFU, TP53 mutation	TERT promoter mutations	MYC, OTX2, SMARCA4, NOTCH, TGF-β mutations		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% (α), 100% (β)	69.8% (α), 67.3% (β), 88% (γ), 88.5% (δ)		50% (II) 43% (III) 80% (IV)	77% (I) 59% (V) 85% (VII)	81% (VI) 81% (VIII)	
Metastasis (%)	12%	20%(α), 33%(β), 9% (γ), 9% (δ)		57% (II) 56% (III) 58% (IV)	35% (I) 62% (V) 45% (VII)	45% (VI) 50% (VIII)	

Other CNS Embryonal Tumors

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)

- 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

Molecular subgroups	*AT/RT-SHH*	*AT/RT-TYR*	*AT/RT-MYC*
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 mjutations/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

- 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

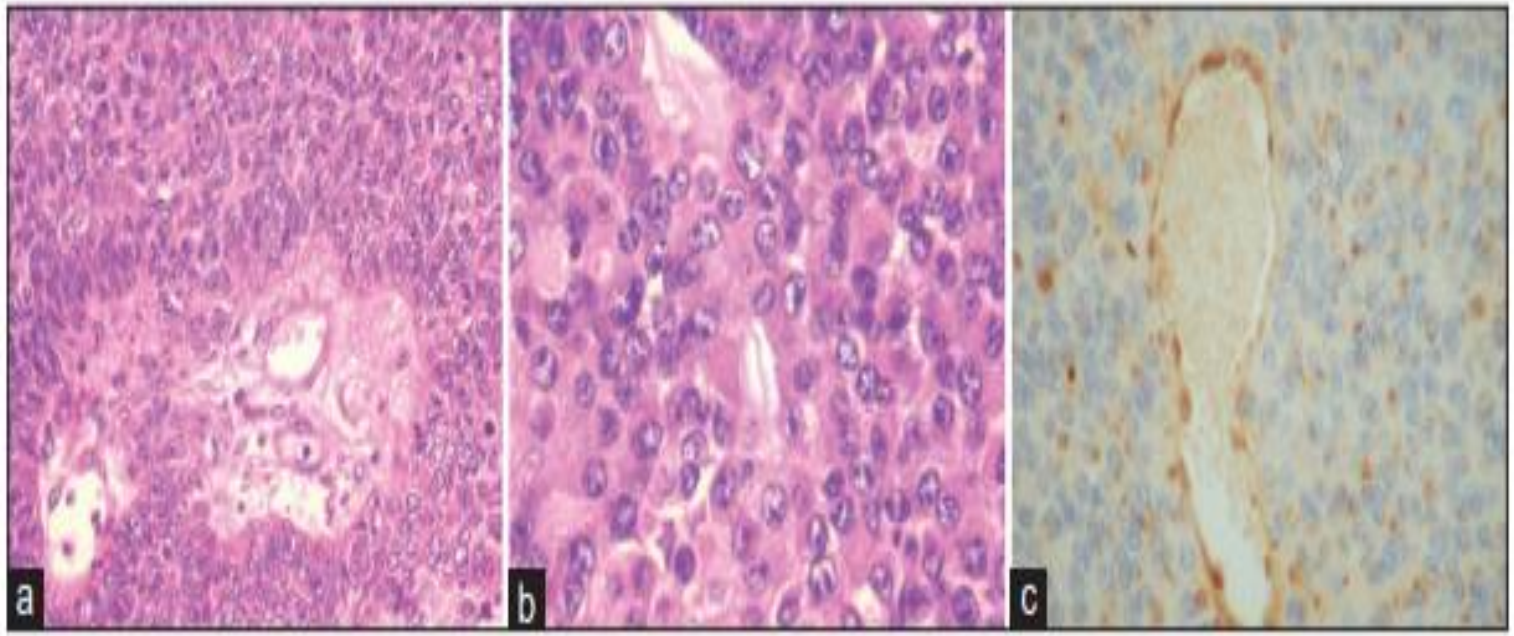

Figure 3: ATRT. (a) H and E. Neoplasticrhabdoidcells display a perivascular arrangement (20 ×). (b) The tumorhas medium-sized, round cells with distinct borders, eccentric nuclei, and prominent nucleoli (H and E) (40 ×). (c) INI1 immunostainshowing positive endothelial and reactivecells. Tumorcells are negative for INI1 protein (20X)

Cribriform Neuroepithelial Tumor

- 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)

- 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)

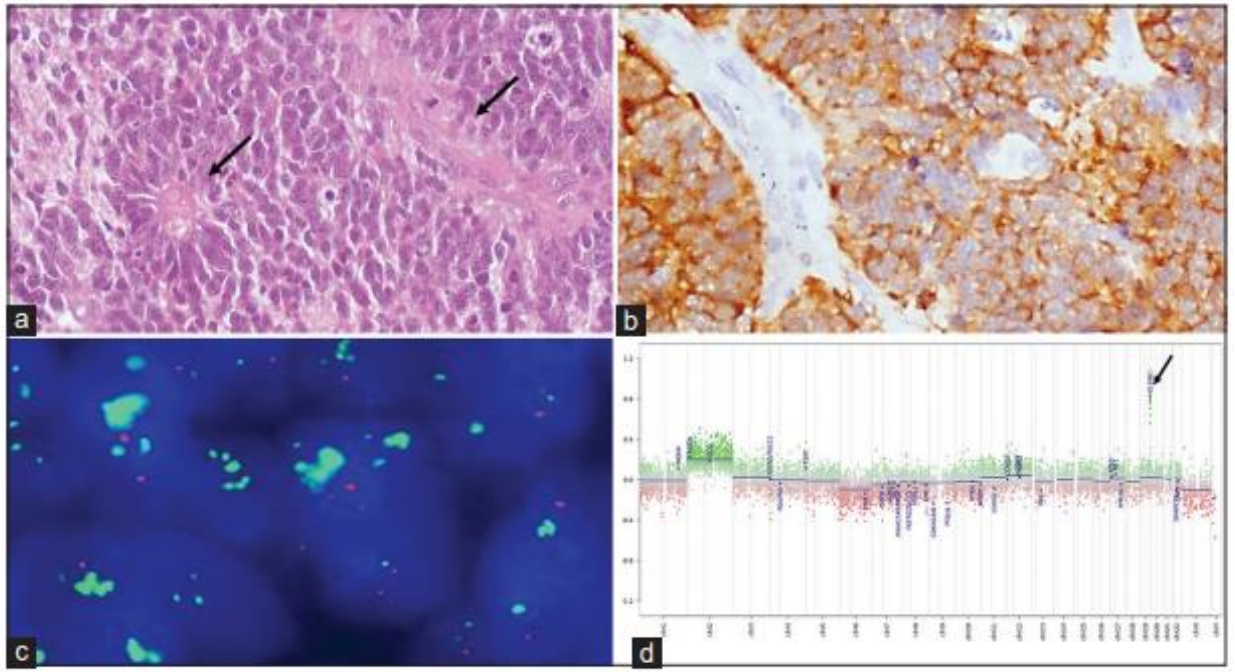

Figure 4: ETMR. (a) H and E. Neoplastic cells with hyperchromatic nuclei. Multilayeredrosettes (arrows) are identified. (20 ×). (b) Immunohistochemistry for LIN28 showing strong cytoplasmic stain (20 ×). (c) FISH shows amplification at 19q13.42 (green signals). (d) Copy number variation analysis with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

FOXR2-Activated CNS Neuroblastoma (CNS NB-FOXR2)

- 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)

- Immunoprofile:

Strong OLIG2 positivity.

Negative for GFAP and vimentin.

Areas of neuronal differentiation positive for synaptophysin.

Most cases show TTF-1 overexpression

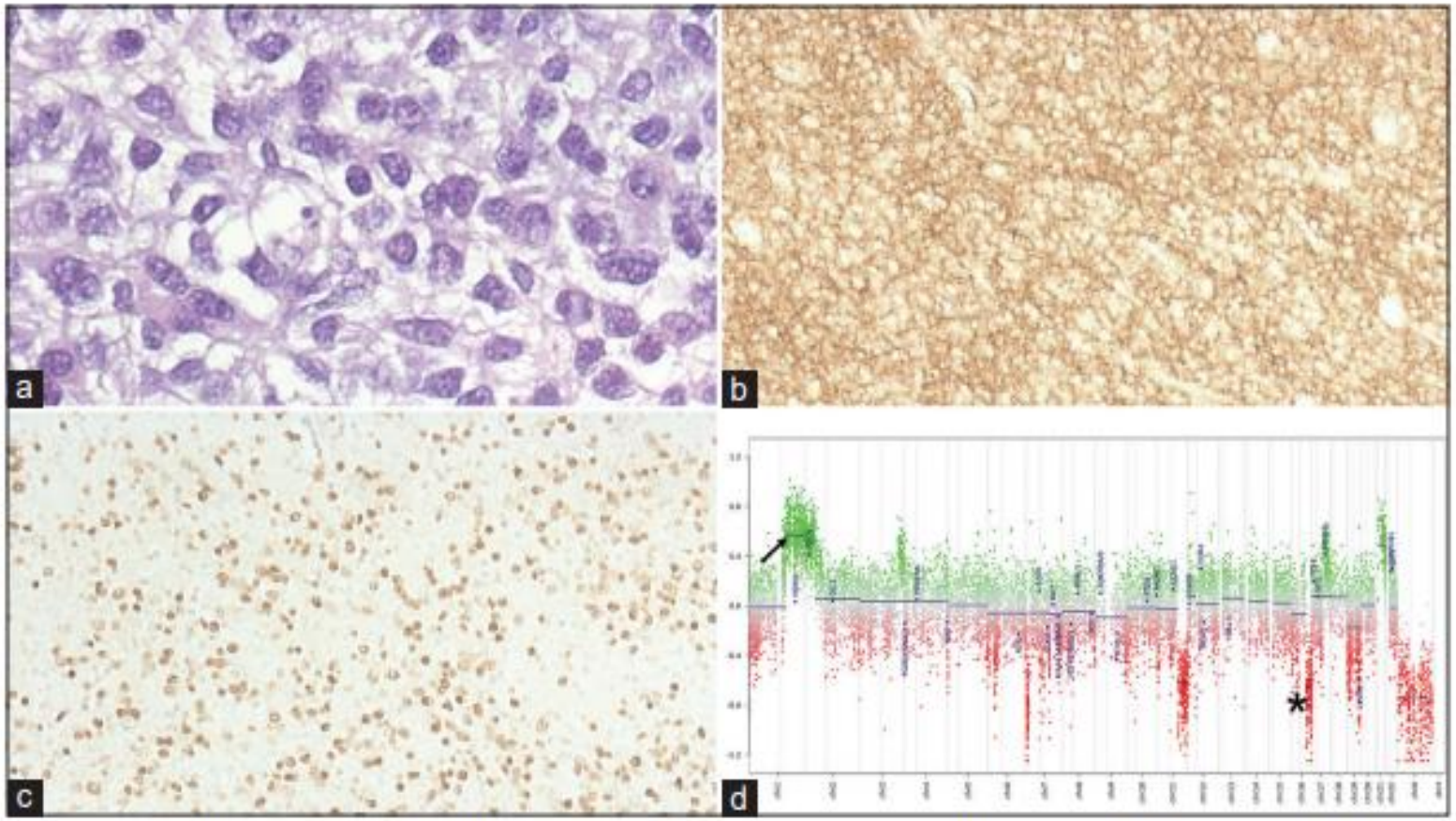

Figure 5: CNS neuroblastoma, FOXR2-activated. (a) H and E. Neoplasticcells with small, round nuclei surrounded by a clear halo (40 ×). (b) Synaptophysin immunoexpression (10 ×). (c) Olig2 immunoexpression (10 ×). (d) Copy number variation. Gain of chromosome 1q (arrow) and focal or total loss of 16q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

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

- 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

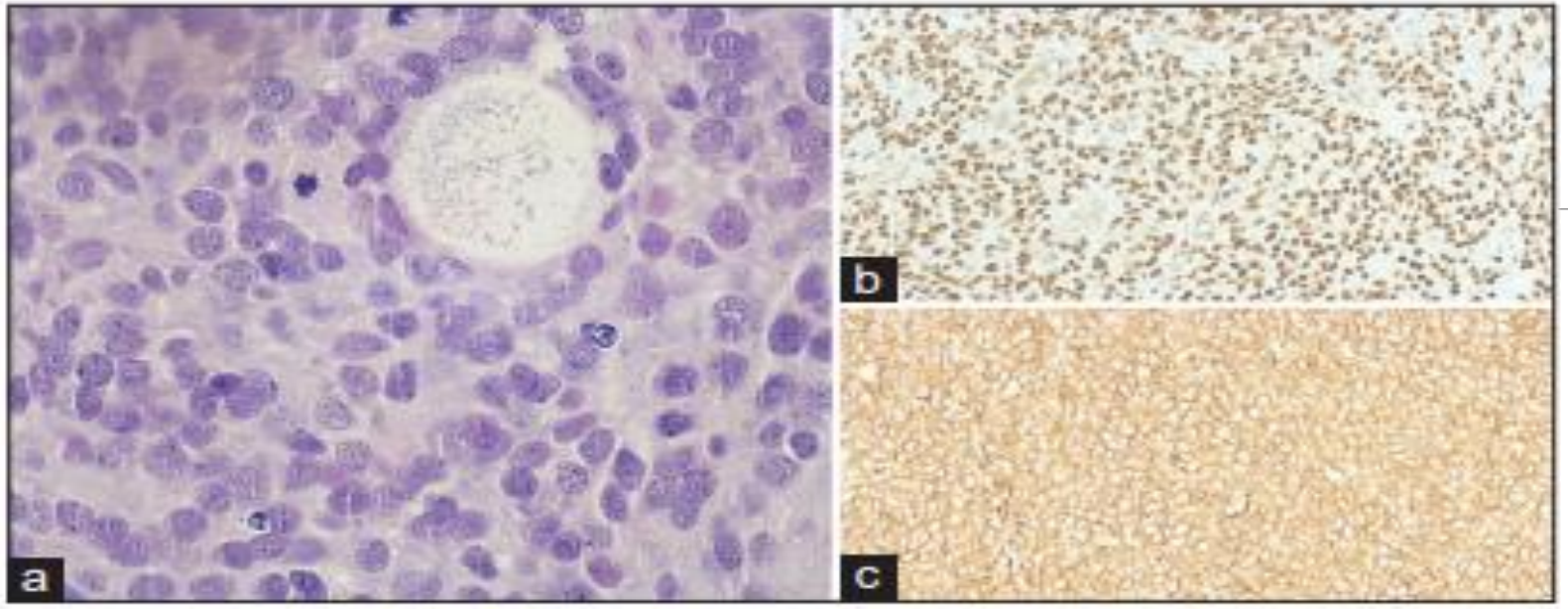

Figure 6: CNS tumor with BCOR internal tandem duplication (ITD). (a) H and E. Tumor cells show an oligo-like aspect with monotonous round to oval nuclei, fine chromatin, and indistinct nucleoli. Evident some microcystic formation (40 ×). (b) Nuclear BCOR immunostaining (10 ×). (c) Diffuse immunopositivity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

CNS Tumor with BCOR-ITD

- 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

- 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

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.

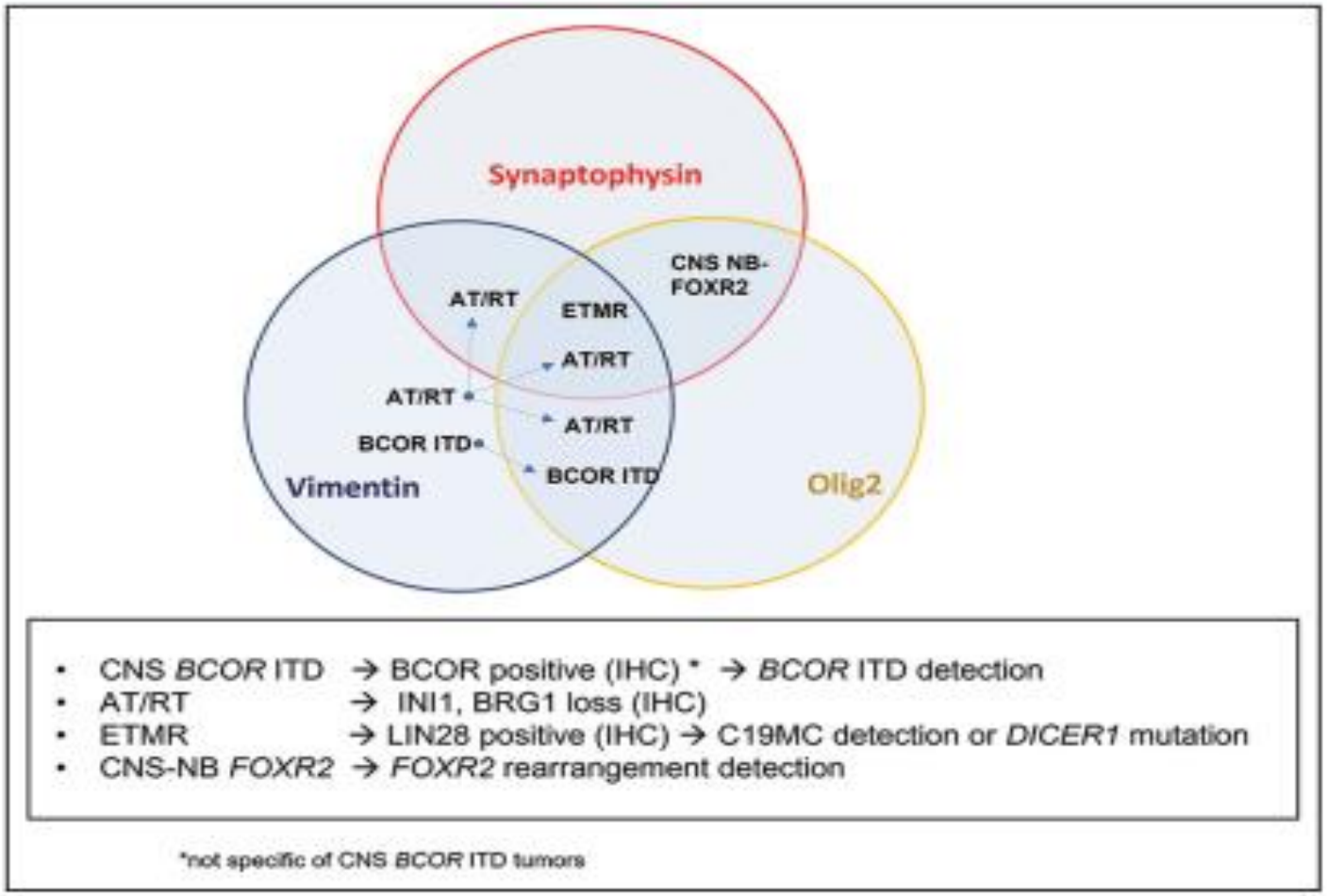

Figure 7: Diagnostic algorithm for approaching diagnosis of non-medulloblastoma embryonal tumors, including the integration of immunohistochemical and molecular analysis

Thank You