

Tumours of the Central Nervous System Molecular Information Reporting Guide

Family/Last name

Date of birth

DD – MM – YYYY

Given name(s)

Patient identifiers

Date of request

DD – MM – YYYY

Accession/Laboratory number

Elements in **black text** are **CORE**. Elements in **grey text** are **NON-CORE**.☐ indicates multi-select values ☐ indicates single select values

SCOPE OF THIS DATASET SECTION

ADEQUACY OF SPECIMEN FOR MOLECULAR ASSESSMENT ^(Note 1)

- ☐ Specimen is adequate for analysis
☐ Specimen is inadequate for analysis (select all that apply)

- ☐ Crush
☐ Autolysis
☐ Cautery
☐ Necrosis
☐ Decalcification
☐ Tumour cell quantity
☐ Fixation issues, *specify*

☐ Other, *specify*

Representative blocks for ancillary studies, specify those blocks best representing tumour and/or normal tissue for further study

ALK/ROS1/MET/NTRK FAMILY ALTERATIONS^a (Note 2)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD^b (select all that apply)

- ☐ Immunohistochemistry (IHC)
☐ In situ hybridisation (ISH)
☐ Next generation sequencing (NGS)
☐ Other, *specify*

^a Only core for some tumours - refer to [Tables 1-3](#).^b Repeat for each alteration.

ATRX ALTERATIONS^a (Note 3)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ IHC
☐ Indeterminate
☐ Intact nuclear expression
☐ Loss of nuclear expression

☐ Other, *specify*

BCOR INTERNAL TANDEM DUPLICATION^a (Note 4)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

BRAF ALTERATIONS^a (Note 5)

BRAF variant

- ☐ Indeterminate
☐ Absent
☐ BRAF p.V600E (c.1799T>A) variant present
☐ Other BRAF sequence variant present, *specify*

VARIANTS ASSESSED (select all that apply)

- ☐ p.V600E
☐ Any variant in exon 15
☐ Other BRAF variant present, *specify*

BRAF ALTERATIONS^a (Note 5) continued

BRAF variant continued

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ IHC

BRAF p.V600E expression

- ☐ Indeterminate
☐ Negative
☐ Positive

☐ Other, *specify*

BRAF rearrangement/duplication

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

VARIANTS ASSESSED (select all that apply)

- ☐ KIAA1549::BRAF fusion
☐ BRAF::RAF1 fusion
☐ Other, *specify*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ RT-PCR
☐ Array-based method
☐ RNA-sequencing
☐ Other, *specify*

^a Only core for some tumours - refer to [Tables 1-3](#).

C19MC ALTERATIONS^a (Note 6)

- ☐ Indeterminate
☐ Absent
☐ Present with low level gain
☐ Present, *describe including copy number*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ Array-based method
☐ NGS
☐ Other, *specify*

LIN28A expression (IHC)^a

- ☐ Indeterminate
☐ Negative
☐ Positive

CDKN2A/B DELETION^a (Note 7)

- ☐ Indeterminate
☐ Absent
☐ Homozygous deletion
☐ Hemizygous/heterozygous deletion

TESTING METHOD (select all that apply)

- ☐ ISH
☐ Array-based method
☐ NGS
☐ Other, *specify*

WHOLE CHROMOSOMAL ARM 1p/19q CODELETION^a (Note 8)

- ☐ Indeterminate
☐ None detected
☐ 1p/19q codeletion
☐ 1p only deletion
☐ 19q only deletion
☐ Polysomy, *specify*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ Array-based method
☐ PCR/Loss of heterozygosity assay
☐ NGS
☐ Methylome profiling
☐ Other, *specify*

WHOLE CHROMOSOME 7 GAIN (COMBINED WITH WHOLE CHROMOSOME 10 LOSS)^a (Note 9)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ Array-based method
☐ NGS
☐ Other, *specify*

CIC ALTERATIONS^a (Note 10)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

DICER1 ALTERATIONS^a (Note 11)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

^a Only core for some tumours - refer to [Tables 1-3](#).

EGFR ALTERATIONS^a (Note 12)

EGFR amplification

- ☐ Indeterminate
☐ Absent
☐ Absent with low level gain
☐ Present, *describe including copy number*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ Array-based method
☐ NGS
☐ Other, *specify*

EGFRvIII variant

- ☐ Indeterminate
☐ Absent
☐ Present

TESTING METHOD (select all that apply)

- ☐ NGS
☐ PCR-based method
☐ IHC
☐ Other, *specify*

FET ALTERATIONS^a (Note 13)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

FGFR FAMILY ALTERATIONS^a (Note 14)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD^b (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

^b Repeat for each alteration.

FOXR2 ALTERATIONS^a (Note 15)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

HISTONE H3 VARIANTS AND LOSS OF H3 p.K28me3 (K27me3)^a (Note 16)

Histone H3 gene family variants

- ☐ Indeterminate
☐ Negative
☐ Positive for K27M
☐ Positive for G34R or G34V
☐ Positive, for other H3 variants, *specify*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ IHC

Histone H3 K27M expression

- ☐ Indeterminate
☐ Negative
☐ Positive

Histone H3 G34R expression

- ☐ Indeterminate
☐ Negative
☐ Positive

Histone H3 K27me3 expression

- ☐ Indeterminate
☐ Intact expression
☐ Loss of expression

☐ Other, *specify*

IDH ALTERATIONS^a (Note 17)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ IHC

IDH1 R132H expression

- ☐ Indeterminate
☐ Negative
☐ Positive

☐ Other, *specify*

^a Only core for some tumours - refer to [Tables 1-3](#).

MAPK PATHWAY ALTERATIONS (Note 18)

- ☐ Indeterminate
☐ Absent
☐ NF1 loss, *describe*

For *BRAF-KIAA1549* alterations refer to **BRAF ALTERATIONS** (Note 5)

For *FGRFR* alterations refer to **FGFR FAMILY ALTERATIONS** (Note 14)

☐ Positive for other MAPK alteration, *describe*

TESTING METHOD^b (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

^b Repeat for each alteration.

METHYLOME PROFILING^a (Note 19)

Classifier (e.g., Heidelberg Brain Tumour Classifier)	Version (e.g., 12.5)	Indicated diagnosis	Score

MGMT promoter status

- ☐ Indeterminate
☐ Unmethylated
☐ Methylated

Most informative copy number variations, *specify*

MN1 ALTERATIONS^a (Note 20)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

MYB, MYBL1 ALTERATIONS^a (Note 21)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD^b (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

MYC GENE FAMILY AMPLIFICATION (MYC and/or MYCN)^a (Note 22)

- ☐ Indeterminate
☐ Absent
☐ Absent with low level gain
☐ Present, *describe including copy number*

TESTING METHOD^b (select all that apply)

- ☐ ISH
☐ Array-based method
☐ NGS
☐ Other, *specify*

PDGFRA ALTERATIONS^a (Note 23)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

PITUITARY HORMONES AND TRANSCRIPTION FACTORS IMMUNOHISTOCHEMISTRY^a (Note 24)

Tumour cells are reactive for (select all that apply)

- ☐ Indeterminate
☐ Prolactin
☐ Human growth hormone
☐ β-TSH
☐ β-FSH
☐ β-LH
☐ Alpha subunit
☐ ACTH
☐ PIT1
☐ TPIT
☐ SF1
☐ Other, *specify*

^a Only core for some tumours - refer to [Tables 1-3](#).

PRC2 INACTIVATION^a (Note 25)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

PRKAR1A INACTIVATION^a (Note 26)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

PRKCA INACTIVATION^a (Note 27)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

SHH PATHWAY ALTERATIONS^a (Note 28)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD^b (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

^b Repeat for each alteration.

SMARC FAMILY ALTERATIONS^a (Note 29)

SMARCA4/BRG1 alteration

- ☐ Indeterminate
☐ Absent
☐ Present, *describe sequence variant(s)*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ Other, *specify*

BRG1 loss of expression (IHC)

- ☐ Indeterminate
☐ Intact nuclear expression
☐ Loss of nuclear expression

SMARCB1/INI1/HSNF5 alteration

- ☐ Indeterminate
☐ Absent
☐ Present, *describe sequence variant(s)*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ Other, *specify*

INI1 (BAF47) loss of expression (IHC)

- ☐ Indeterminate
☐ Intact nuclear expression
☐ Loss of nuclear expression

STAT6 IMMUNOHISTOCHEMISTRY AND REARRANGEMENT^a (Notes 30)

STAT6 expression (IHC)

- ☐ Indeterminate
☐ Absence of nuclear expression
☐ Positive nuclear expression

STAT6 rearrangement

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ NGS
☐ Other, *specify*

^aOnly core for some tumours - refer to [Tables 1-3](#).

TERT PROMOTER ALTERATIONS^a (Note 31)

- ☐ Indeterminate
☐ Absent
☐ Hotspot variant (C228T or C250T)
☐ Other sequence variant, *specify*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ Other, *specify*

TP53 ALTERATIONS^a (Note 32)

TP53 variant

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

EXONS ANALYSED

- ☐ Exons 5-8
☐ All exons
☐ Other, *specify*

TESTING METHOD (select all that apply)

- ☐ Sanger sequencing
☐ NGS
☐ PCR-based method
☐ IHC

p53 expression

- ☐ Negative or rare, lightly positive cells
☐ Intermediate (intermediate numbers of predominantly lightly positive cells)
☐ Positive (diffuse and strong nuclear positivity)

☐ Other, *specify*

TTF1 EXPRESSION (IHC)^a (Note 33)

- ☐ Indeterminate
☐ Negative
☐ Positive

WNT PATHWAY ALTERATIONS^a (Note 34)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD^b (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

^b Repeat for each alteration.

YAP1 REARRANGEMENT^a (Note 35)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ ISH
☐ NGS
☐ Other, *specify*

ZFTA REARRANGEMENT^a (Note 36)

- ☐ Indeterminate
☐ Absent
☐ Present, *describe*

TESTING METHOD (select all that apply)

- ☐ IHC
☐ ISH
☐ NGS
☐ Other, *specify*

L1CAM expression (IHC)

- ☐ Indeterminate
☐ Negative
☐ Positive

ZFTA REARRANGEMENT^a (Note 36) continued

RELA rearrangement

- ☐ Indeterminate
- ☐ Absent
- ☐ Present, describe

TESTING METHOD (select all that apply)

- ☐ ISH
- ☐ NGS
- ☐ Other, specify

^a Only core for some tumours - refer to Tables 1-3.

OTHER IMMUNOHISTOCHEMISTRY FINDINGS^a (Note 37)

- ☐ None identified
- ☐ Present, specify test, testing method and findings

OTHER MOLECULAR FINDINGS (Note 38)

- ☐ None identified
- ☐ Present, specify test, testing method and findings

Definitions

CORE elements

Core elements are those which are essential for the clinical management, staging or prognosis of the cancer. These elements will either have evidentiary support at Level III-2 or above (based on prognostic factors in the National Health and Medical Research Council levels of evidence¹). In rare circumstances, where level III-2 evidence is not available an element may be made a core element where there is unanimous agreement by the Dataset Authoring Committee (DAC).

The summation of all core elements is considered to be the minimum reporting standard for a specific cancer.

Non-morphological testing e.g., molecular or immunohistochemical testing is a growing feature of cancer reporting. However, in many parts of the world this type of testing is limited by the available resources. In order to encourage the global adoption of ancillary tests for patient benefit, International Collaboration on Cancer Reporting (ICCR) includes the most relevant ancillary testing in ICCR Datasets as core elements, especially when they are necessary for the diagnosis. Where the technical capability does not yet exist, laboratories may consider temporarily using these data elements as non-core items.

NON-CORE elements

Non-core elements are those which are unanimously agreed should be included in the dataset but are not supported by level III-2 evidence. These elements may be clinically important and recommended as good practice but are not yet validated or regularly used in patient management.

Key information other than that which is essential for clinical management, staging or prognosis of the cancer such as macroscopic observations and interpretation, which are fundamental to the histological diagnosis and conclusion e.g., macroscopic tumour details, may be included as either core or non-core elements by consensus of the DAC.

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Scope

This dataset section has been developed for the molecular assessment of primary central nervous system (CNS) tumours, whether that molecular assessment is nucleic acid or protein-based. This section does not apply to those tumours in which molecular information is not captured for diagnostic purposes. However, as this dataset section applies to a growing subset of CNS tumours, it is anticipated that its use will increase over time.

This dataset section on molecular assessment should be used in conjunction with the dataset sections on 'Histological assessment of CNS specimens' and the 'Final integrated report/diagnosis for CNS specimens'.

The 2nd edition of this dataset incorporates the World Health Organization (WHO) Classification of Tumours of the CNS, 5th edition (CNS5), 2021.² A complete diagnosis of CNS tumours should ideally conform to the final integrated diagnoses in the 2021 WHO CNS5 Tumour Classification, which for most tumour types now require integration of elements from histological and ancillary analyses.

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Overview of selected molecular (including protein) diagnostic markers for CNS tumours

Tables 1-3 describe the molecular and immunohistochemistry (IHC) markers listed as essential or desirable criteria for tumours in the WHO CNS5 Tumour Classification.² The tables should be used as a reference to determine which markers are core (in bold) or non-core (non-bold) for each tumour entity. Molecular and IHC alterations which are core versus non-core correspond to WHO essential and desirable diagnostic criteria, respectively.

While these elements are deemed core, in some jurisdictions, consideration should be given to temporarily downgrading them to a non-core element until resources allow.

The list of alterations is not exhaustive and other markers or assays may be helpful in some diagnostic circumstances. In addition, the tests listed are mostly related to 'ruling in' the corresponding diagnoses. However, it should be realised that the assays may also be used in particular diagnostic situations to 'rule out' other diagnoses. An example of this would be ATRX IHC, which is commonly used to support a diagnosis of IDH-mutant astrocytoma, but which is also used to evaluate a possible diagnosis of oligodendroglioma, IDH-mutant and 1p/19q-codeleted.

Some specific alterations recommended in the commentaries below represent one of several validated and equivalent approaches to the evaluation of the described molecular variable. For those alterations that have multiple testing modalities (e.g., sequencing for *BRAF* p.V600E and IHC for the mutant protein), it is assumed that only one of these testing modalities would be used per case unless one test yields equivocal results (e.g., a result of weak IHC positivity versus nonspecific background staining should be followed by gene sequencing). For some tests, relevance may be related to the age of the patient (e.g., *EGFR* gene amplification in IDH-wildtype glioblastoma rather than in paediatric-type high grade gliomas). The reader is referred to the commentaries under each molecular parameter for further information.

In many instances in this dataset, the term 'variant' or 'sequence alteration' is used in place of the term 'mutation', based on the consensus recommendations of the American College of Medical Genetics and Genomics, the Association for Molecular Pathology, the Clinical Genome Resource, Cancer Genomics Consortium and the Variant Interpretation for Cancer Consortium.^{3,4}

The use of published algorithms may be helpful in some situations, such as for [molecular biomarker testing for the diagnosis of diffuse gliomas](#).⁵ Diagnostic algorithms can be beneficial to assist with stepwise decisions, especially when resources are limited. However, diagnostic algorithms can be overly rigid and have the potential to skew usage towards select molecular assays in place of acceptable alternative surrogate markers.

Table 4 describes genetic CNS tumour syndromes summarised from the WHO CNS5 Tumour Classification.²

Table 1. Molecular and immunohistochemical alterations listed as essential or desirable diagnostic criteria for gliomas and glioneuronal tumours in the 2021 World Health Organization Classification of Tumours of the Central Nervous System.^{2#}

Note: Alterations in **bold** correspond to core ICCR/essential WHO criteria; non-bold alterations correspond to non-core ICCR/desirable WHO criteria.

Note: Refer to the hyperlinked specific notes for further details on core criteria.

TUMOUR FAMILY/tumour type	Alterations
ADULT-TYPE DIFFUSE GLIOMAS	
Astrocytoma, IDH-mutant	(IDH1 p.R132 ^a or IDH2 p.R172 ^a) & (ATRX ^{b,c} or exclusion of chr 1p/19q ^d); [CDKN2A/B ^e]; <i>TP53</i> , ^{c,f} MP
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	(IDH1 p.R132 ^a or IDH2 p.R172 ^a) & chr 1p/19q ^d MP, <i>ATRX</i> , ^f <i>TERT</i> promoter ^c
Glioblastoma, IDH-wildtype	IDH ^{wt} / H3 ^{wt} & (TERT promoter ^c or EGFR ^g or chr +7/-10 ^h); MP; [<i>MGMT</i> promoter methylation]
PAEDIATRIC-TYPE DIFFUSE LOW GRADE GLIOMAS	
Diffuse astrocytoma, <i>MYB</i> - or <i>MYBL1</i> -altered	(Absence of IDH ^c or H3 ^c) & (MYB ⁱ / MYBL1 ⁱ or MP); absence of <i>OLIG2</i> ^f and <i>MAP2</i> ^f
Angiocentric glioma	<i>MYB</i> ; ⁱ MP
Polymorphous low grade neuroepithelial tumour of the young (PLNTY)	CD34 ^f & IDH ^{wt} & (BRAF p.V600 ^{f,j} or FGFR2 ^k / FGFR3 ^k or other MAPK pathway alteration); absence of 1p/19q ^d ; [MP]
Diffuse low grade glioma, MAPK pathway-altered	MAPK pathway alteration & IDH ^{wt} & H3 ^{wt} & absence of CDKN2A ; ^e MP
PAEDIATRIC-TYPE DIFFUSE HIGH GRADE GLIOMAS	
Diffuse midline glioma, H3K27-altered	Loss of H3 p.K28me3 (K27me3) ^b & (H3 p.K28M (K27M) ^c /pK28I (K27I) ^c or EGFR ^{c,g} or EZH1 ⁱ or MP); discrimination of H3.1 or H3.2 versus H3.3 p.K28 (K27)-mutant subtypes
Diffuse hemispheric glioma, H3G34-mutant	H3F3A p.G35 (G34) ^j & (for unresolved lesions) MP; negative <i>OLIG2</i> ; ^f <i>ATRX</i> , ^b p53 ^f
Diffuse paediatric-type high grade glioma, H3- and IDH-wildtype	(Absence of IDH1 ^c or IDH2 ^c) & absence of H3 ^c & (MP or PDGFRA ^{c,g} / EGFR ^{c,g} / MYCN ^g); H3 p.K28me3 (K27me3) retained
Infant-type hemispheric glioma	RTK/NTRK family member abnormality (e.g., <i>ROS1</i> , ^k <i>MET</i> , ^k <i>ALK</i> ^k) or MP
CIRCUMSCRIBED ASTROCYTIC GLIOMAS	
Pilocytic astrocytoma	MAPK alteration, such as <i>BRAF</i> ^{c,j,k} (most frequent <i>KIAA1549::BRAF</i>), [<i>NF1</i> , ^j <i>FGFR1</i> , ^{j,k} <i>NTRK1/2/3</i> , ^k MP]

High grade astrocytoma with piloid features	MP ; MAPK alteration (e.g., <i>NF1</i> ^{b,c} <i>BRAF</i> ^k esp. <i>KIAA1549::BRAF</i> , <i>FGFR1</i> ^c); <i>CDKN2A/B</i> ^{c,e} or <i>CDK4</i> ^g <i>ATRX</i> ^{b,c}
Pleomorphic xanthoastrocytoma	MAPK alteration (e.g., <i>BRAF</i> p.V600, ^j <i>BRAF</i> ^{c,k} <i>NTRK1/2/3</i> , <i>RAF1</i> , <i>NF1</i>), combined with <i>CDKN2A/B</i> ^e MP
Subependymal giant cell astrocytoma	GFAP ^f , S100 ^f & neuronal markers ^f (e.g., class III β-tubulin ^f , neurofilament ^f , synaptophysin ^f , NeuN ^f); TTF1, ^f tuberlin, ^f harmarin, ^f phosphorylated S6, ^f <i>TSC1</i> ^c or <i>TSC2</i> ^c MP
Chordoid glioma	TTF1, ^f <i>PRKCA</i> p.D463H ^c or MP
Astroblastoma, <i>MN1</i> -altered	MN1 ^h & (for unresolved lesions) MP ; GFAP, ^f EMA ^f , [<i>BEND2</i> ^k]
GLIONEURONAL AND NEURONAL TUMOURS	
Ganglioglioma	BRAF ^{c,j,k} or other MAPK alteration (e.g., <i>RAF1</i> ^k , <i>KRAS</i> ^j , <i>NF1</i> ^{b,j}) or MP (for unresolved lesions); Absence of IDH ^c
Gangliocytoma	-
Desmoplastic infantile ganglioglioma	MP or (RAF ^{c,k} or RAF1 ^{c,k} in the absence of CDKN2A/B ^e)
Dysembryoplastic neuroepithelial tumour	FGFR1 ^{a,k,m} or MP (for unresolved lesions)
Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters*†	MP & OLIG2 ^f & synaptophysin ^f & GFAP ^b ; chr 14 ⁿ
Papillary glioneuronal tumour	PRKCA ^k (mostly <i>SLC44A1::PRKCA</i>) & MP (for unresolved lesions)
Rosette-forming glioneuronal tumour	MP (for unresolved lesions); <i>FGFR1</i> ^c with <i>PIK3CA</i> ^c and/or <i>NF1</i> ^c
Myxoid glioneuronal tumour	<i>PDGFRA</i> p.K385, ^j <i>PDGFRA</i> ^c MP
Diffuse leptomeningeal glioneuronal tumour‡	OLIG2 ^f & synaptophysin ^f & Chr 1p ^d & MAPK alteration, mostly <i>BRAF</i> ^k (such as <i>KIAA1549::BRAF</i>); MP (for unresolved lesions)
Multinodular and vacuolating neuronal tumour	Synaptophysin ^f , HuC/HuD ^f or non-phosphorylated 200kDa NFP ^f ; <i>OLIG2</i> ^f & internexin A, ^f NeuN ^b or chromogranin, ^f MAPK alteration (esp. <i>MAP2K1</i> ^c ; <i>FGFR2</i> ^k , <i>BRAF</i> ^c)
Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)	<i>PTEN</i> ^{b,c}
Central neurocytoma	Synaptophysin ^f & MP (for unresolved lesions)
Extraventricular neurocytoma	Absence of IDH ^h & Synaptophysin ^f & MP (for unresolved lesions); <i>FGFR1</i> alteration (mostly <i>FGFR1::TACC1</i>), [<i>FGFR3</i> ^k]
Cerebellar liponeurocytoma	Synaptophysin ^f & MP (for unresolved lesions); focal GFAP ^f

EPENDYMAL TUMOURS	
Supratentorial ependymoma, <i>ZFTA</i> fusion positive	IHC features of ependymoma & <i>ZFTA</i> (<i>C11orf95</i>)^k (mostly <i>ZFTA::RELA</i>); MP, p65 (<i>RELA</i>) ^f or L1CAM ^f
Supratentorial ependymoma, <i>YAP1</i> fusion positive	IHC features of ependymoma & <i>YAP1</i> ^k ; MP, negative for p65 (<i>RELA</i>) ^f or L1CAM ^f
Posterior fossa ependymoma, group A (PFA)	IHC features of ependymoma & (MP or reduction of H3 p.K28me3 (K27me3)^f in tumour cell nuclei) ; stable genome on genome-wide copy-number analysis
Posterior fossa ependymoma, group B (PFB)	IHC features of ependymoma & MP ; chromosomal instability and aneuploidy on genome-wide copy-number analysis, retained H3 p.K28me3 (K27me3) ^f in tumour cell nuclei
Spinal ependymoma	IHC features of ependymoma ; MP, 22q, ^d absence of <i>MYCN</i> ^g
Spinal ependymoma, <i>MYCN</i> -amplified	IHC features of ependymoma & <i>MYCN</i> ^g ; MP
Myxopapillary ependymoma	GFAP^f & MP (for unresolved lesions)
Subependymoma	MP (for unresolved lesions)

MP – Methylome profiling; IHC – immunohistochemistry; MAPK – Mitogen-activated protein kinase; ^{wt} wildtype; ^a missense mutation/variant; ^b loss of expression; ^c mutation/variant; ^d combined whole-arm deletion; ^e homozygous deletion; ^f expression; ^g amplification; ^h copy number alteration; ⁱ structural variant; ^j hotspot mutation/variant; ^k gene fusion; ^l overexpression; ^m internal tandem duplication; ⁿ monosomy.

Alterations in [brackets] are not derived from the tables of essential or desirable WHO CNS5 Tumour Classification criteria but are considered important predictive or prognostic markers by the ICCR CNS DAC.

* Provisional tumour type; † Methylation profiling is so far the only method to clearly identify diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters, but if not available, morphological features may provide an approximation; ‡ This tumour type shows molecular overlap with pilocytic astrocytoma (*KIAA1549::BRAF* fusion) and oligodendroglioma (1p/19q codeletion). All diffuse leptomeningeal glioneuronal tumours are wildtype in *IDH1* and *IDH2*.

This table does not represent a diagnostic algorithm and one should refer to the WHO CNS5 Tumour Classification on how to use this information. Of note, this list of alterations is not exhaustive, and some of the alterations are generally mutually exclusive (e.g., *IDH1* versus *IDH2* variant), while others can occur in combination in the same tumour (e.g., *TERT* promoter variant, *EGFR* amplification, and +7/-10). Furthermore, while this table lists the alterations, demonstration of lack of particular alteration(s) can also be essential to establish the correct diagnosis (e.g., absence of complete 1p/19q codeletion in *IDH*-mutant astrocytomas).

Modified from Table 1 in Sahm et al. Molecular diagnostic tools for the World Health Organization (WHO) 2021 classification of gliomas, glioneuronal and neuronal tumours; an EANO guideline (licensed under [CC-BY-NC 4.0](#)).⁶

For more information refer to the 2021 WHO CNS5 Tumour Classification.²

Table 2. Molecular and immunohistochemical alterations listed as essential and desirable diagnostic criteria for embryonal tumours in the 2021 World Health Organization Classification of Tumours of the Central Nervous System.²

Note: Alterations in **bold** correspond to core/essential WHO criteria; non-bold alterations correspond to non-core/desirable WHO criteria.

Note: Refer to the hyperlinked specific notes for further details on core criteria.

TUMOUR FAMILY/tumour type	Alterations
MEDULLOBLASTOMA, MOLECULARLY DEFINED	
Medulloblastoma, WNT-activated	WNT pathway activation or MP
Medulloblastoma, SHH-activated and <i>TP53</i> -wildtype	(SHH pathway activation or MP) & TP53 ^{wt}
Medulloblastoma, SHH-activated and <i>TP53</i> -mutant	(SHH pathway activation or MP) & TP53 ^a
Medulloblastoma, non-WNT/non-SHH	No WNT or SHH pathway activation or MP
OTHER CNS EMBRYONAL TUMOURS	
Atypical teratoid/rhabdoid tumour	SMARCB1/SMARCA4 ^b or MP (for unresolved lesions); <i>SMARCB1</i> ^c ; <i>SMARCA4</i> ^c
Cribiform neuroepithelial tumour*	SMARCB1 ^b ; EMA ^d
Embryonal tumour with multilayered rosettes (ETMR)	IHC features of ETMR & (C19MC ^c or DICER1 ^a) & MP (for unresolved lesions)
CNS neuroblastoma, <i>FOXR2</i> -activated	FOXR2 ^{e,f} or MP (for unresolved lesions)
CNS tumour with <i>BCOR</i> internal tandem duplication	BCOR exon 15 ^g & MP (for unresolved lesions)
CNS embryonal tumour, NEC/NOS	Neuronal markers ^d ; absence of glial markers ^d

MP – Methylome profiling; IHC – immunohistochemistry; ETMR – embryonal tumour with multilayered rosettes; ^{wt} wildtype; ^a mutation/variant; ^b loss of expression; ^c alteration; ^d expression; ^e structural variant; ^f gene fusion; ^g internal tandem duplication.

* Provisional tumour type.

This table does not represent a diagnostic algorithm and one should refer to the WHO CNS5 Tumour Classification on how to use this information.²

Table 3. Molecular and immunohistochemical alterations listed as essential or desirable diagnostic criteria for other central nervous system tumours in the 2021 World Health Organization Classification of Tumours of the Central Nervous System.²

Note: Alterations in **bold** correspond to WHO essential criteria; non-bold alterations correspond to WHO desirable criteria.

Note: Refer to the hyperlinked specific notes for further details on core criteria.

TUMOUR FAMILY/tumour type	Alterations
CHOROID PLEXUS TUMOURS	
Choroid plexus papilloma	IHC features of choroid plexus
Atypical choroid plexus papilloma	IHC features of choroid plexus; in select cases: hyperploidy by genome-wide chromosomal copy-number analysis
Choroid plexus carcinoma	IHC features of choroid plexus; <i>TP53</i> , ^a MP, in select cases: demonstration of hypoploidy by genome-wide chromosomal copy-number analysis
CRANIAL & SPINAL NERVE TUMOURS	
Schwannoma	S100 ^b or SOX10 ; ^b absence of lattice-like CD34; ^b SMARCB1 ^c (INI1) or mosaic pattern of SMARCB1 ^b (INI1)
Neurofibroma	S100, ^b lattice-like CD34; ^b p16 ^c
Perineurioma	(EMA ^b or claudin-1 ^b or GLUT1 ^b) & absence of S100 ^b
Hybrid nerve sheath tumours	IHC for intermingled features of two types of benign nerve sheath tumours
Malignant melanotic nerve sheath tumour	(S100/SOX10 ^b & melanocytic markers ^b (e.g., HMB45 , melan-A) or PRKAR1A2 ^{a,c}) & MP (for unresolved lesions)
Malignant peripheral nerve sheath tumour	(No more than focal/patchy S100/SOX10 ^b & absence of <i>SS18::SSX1/SSX2/SSX3</i> ^{d#}) or PRC2 inactivation (molecularly or via H3 p.K28me3 ^c) or MP (for unresolved lesions); H3 pK28me3, ^c neurofibromin ^c
Cauda equina neuroendocrine tumour (previously paraganglioma)	Synaptophysin ^b or chromogranin ^b & MP (for unresolved lesions); S100, ^b cytokeratin, ^b reticulin silver stain
GERM CELL TUMOURS	
Mature teratoma	-
Immature teratoma	-
Teratoma with somatic-type malignancy	-

Germinoma	OCT4 ^b & KIT ^b (or podoplanin ^b (D2-40) or absence of 5-methylcytosine ^b), absence of CD30 ^b , absence of AFP ^b , hCG ^b
Embryonal carcinoma	CD30 ^b & OCT4 ^b , absent or focal KIT ^b , absence of hCG ^b , absence of AFP ^b , cytokeratin ^b
Yolk sac tumour	AFP ^b , absent or focal KIT ^b , absent or focal CD30 ^b , absence of β-hCG ^b
Choriocarcinoma	β-hCG ^c , absence of KIT ^b (or absence of podoplanin ^b (D2-40)), absence of AFP ^b , absence of OCT4 ^b
Mixed germ cell tumour	-
MELANOCYTIC TUMOURS	
Meningeal melanocytosis/meningeal melanomatosis	<i>NRAS</i> ^a , rarely <i>BRAF</i> ^a
Meningeal melanocytoma/meningeal melanoma	<i>GNAQ</i> ^a , <i>GNA11</i> ^a , <i>PLCB4</i> ^a or <i>CYSLTR2</i> ^a ; <i>SF3B1</i> ^a ; <i>EIFAX</i> ^a or <i>BAP1</i> ^a ; Chr 3; ^e complex copy-number variations
MENINGIOMAS	
Meningioma	[TERT promoter; ^a CDKN2A/B ^c]; MP ; biallelic inactivation of NF2 (or <i>TRAF7</i> , <i>AKT1</i> , <i>KLF4</i> , <i>SMO</i> , <i>PIK3CA</i> , <i>SMARCE1</i> , <i>BAP1</i>); EMA; ^b <i>SSTR2A</i> ^b , chr. 22/22q, ^f loss of chr. 1p, chr. 6, chr. 10q, chr. 14q, chr. 18
MESENCHYMAL, NON-MENINGOTHELIAL TUMOURS	
Solitary fibrous tumour	STAT6 ^b , <i>NAB2::STAT6</i> ^d
Hemangioblastoma	Inhibin ^b , loss or inactivation of <i>VHL</i> ^{a,c,#} , absence of IHC staining for markers of renal cell carcinoma
Intracranial mesenchymal tumour, <i>FET::CREB</i> fusion-positive	FET::CREB ^d , CD99 ^b , EMA ^b , desmin ^b
<i>CIC</i> -rearranged sarcoma	CIC ^d & CD99 ^b & ETV6 ^b , WT1 ^b , MP
Primary intracranial sarcoma, <i>DICER1</i> -mutant	DICER1 ^a , MP (for unresolved lesions)
Ewing sarcoma	CD99 ^b & FET::ETS ^d , NKX2-2 ^b , PAX7 ^b
Chordoma	Brachyury ^b & SMARCB1 ^c (<i>INI1</i>)
PINEAL TUMOURS	
Pineocytoma	IHC for pineal parenchymal differentiation (e.g., synaptophysin ^b)
Pineal parenchymal tumour of intermediate differentiation	IHC for pineal parenchymal differentiation (e.g., synaptophysin ^b) & MP (for unresolved lesions); <i>KBTBD4</i> ^g
Pineoblastoma	<i>SMARCB1</i> ^b (<i>INI1</i>), MP
Papillary tumour of the pineal region	Characteristic IHC (e.g., cytokeratins ^b , SPDEF ^b , CD56 ^b) & MP

Desmoplastic myxoid tumour of the pineal region, <i>SMARCB1</i> -mutant*	SMARCB1 ^c & MP (for unresolved lesions)
TUMOURS OF THE SELLAR REGION	
Adamantinomatous craniopharyngioma	β -catenin; ^b <i>CTNNB1</i> ; ^a absence of <i>BRAF</i> p.V600E ^a
Papillary craniopharyngioma	<i>BRAF</i> p.V600E; ^{a, b} absence of β -catenin; ^b absence of <i>CTNNB1</i> ^a
Pituicytoma	TTF1 ^b & absence of pituitary hormone ^b and hormone transcription factor ^b & absence of neuronal and neuroendocrine marker ^b
Granular cell tumour of the sellar region	TTF1 ^b & absence of pituitary hormone ^b and hormone transcription factor ^b & absence of neuronal and neuroendocrine marker ^b ; ^b CD68 ^b or α 1-antitrypsin ^b
Spindle cell oncocytoma	TTF1 ^b & absence of pituitary hormone ^b and hormone transcription factor ^b & absence of neuronal and neuroendocrine marker ^b ; antimitochondrial antigen ^b
Pituitary adenoma/Pituitary neuroendocrine tumour	IHC for pituitary hormones and/or lineage-specific transcription factors ; Ki-67 ^b (MIB1), [cytokeratins]
Pituitary blastoma	DICER1 ^h

MP – Methylome profiling; IHC – immunohistochemistry; ^a mutation/variant; ^b expression; ^c loss of expression; ^d gene fusion; ^e monosomy; ^f copy number alteration; ^g insertion; ^h alteration.

Alterations in [brackets] are not derived from the tables of essential or desirable WHO CNS5 Tumour Classification criteria but are considered important predictive or prognostic markers by the ICCR CNS DAC.

* Provisional tumour type, [#] [VHL](#) and absence of [SS18::SSX1/SSX2/SSX3](#) have been designated non-core alterations by the ICCR CNS DAC.

This table does not represent a diagnostic algorithm and one should refer to the WHO CNS5 Tumour Classification on how to use this information.²

Table 4. Genetic central nervous system (CNS) tumour syndromes and alterations from the 2021 World Health Organization Classification of Tumours of the CNS.²

Tumour of the (central) nervous system	Genetic tumour syndrome	Gene alteration
Neurofibroma; ANNUBP; hybrid neurofibroma/schwannoma; MPNST; pilocytic astrocytoma; other gliomas	Neurofibromatosis type 1	<i>NF1</i> ^a
(Bilateral) schwannoma; meningioma; spinal ependymoma; hybrid neurofibroma/schwannoma;	Neurofibromatosis type 2	<i>NF2</i> ^a
Schwannoma; meningioma; hybrid neurofibroma/schwannoma; MPNST	Schwannomatosis	Chr. 22; ^{b,c} two <i>NF2</i> ; ^a <i>SMARCB1</i> ^a or <i>LZTR1</i> ^a
Hemangioblastoma	Von Hippel-Lindau syndrome	<i>VHL</i> ^a
Subependymal giant cell astrocytoma	Tuberous sclerosis	<i>TSC1</i> ^a or <i>TSC2</i> ^a
Choroid plexus carcinoma; IDH-wildtype glioblastoma; IDH-mutant astrocytoma, medulloblastoma	Li-Fraumeni syndrome	<i>TP53</i> ^{a,d,e}
Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)	Cowden syndrome	Without known family history of <i>PTEN</i> ^a
IDH-wildtype high grade glioma; IDH-mutant astrocytoma; medulloblastoma	CMMRD syndrome, Lynch syndrome	<i>PMS2</i> ^{a,e} or <i>MSH1</i> ^{a,e} or <i>MSH6</i> ^{a,e} or <i>MLH1</i> ^{a,e} genomic profiling of MMRD; absence of MMR ^f
Medulloblastoma, WNT-activated	Familial adenomatous polyposis 1	<i>APC</i> ; ^a absence of <i>CTNNB1</i> ^a
Medulloblastoma, SHH-activated	Nevoid basal cell carcinoma syndrome	<i>PTCH1</i> ^a or <i>SUFU</i> ^a
Atypical teratoid/rhabdoid tumour	Rhabdoid tumour predisposition syndrome	<i>SMARCB1/A4</i> ^a
Malignant melanotic nerve sheath tumour	Carney complex	Inactivating <i>PRKAR1A</i> ^a
Pituitary blastoma; pineoblastoma; ciliary body medulloepithelioma; DICER1-associated CNS sarcoma; ETMR-like infantile cerebellar embryonal tumour	DICER1 syndrome	<i>DICER1</i> ; ^a <i>DICER</i> ^{a,c} involving remaining allele
Paraganglioma	Familial paraganglioma syndrome	Germline susceptibility gene variant; <i>SDHB</i> ^g (high predictive value for <i>SDHB/C/D</i> ^a)

Pleomorphic xanthoastrocytoma, low grade diffuse astrocytoma; IDH—wildtype glioblastoma; schwannoma; neurofibroma	Melanoma-astrocytoma syndrome	<i>CDKN2A</i> ^a (heterozygous); somatic inactivation of remaining <i>CDKN2A</i> ^{wt} with accompanying somatic alterations (e.g., <i>BRAF</i> pV600E, ^a <i>IDH1</i> ^a or <i>IDH2</i> ^a)
Retinoblastoma; pineoblastoma	Familial retinoblastoma	<i>RB1</i> ^h
Meningioma, rhabdoid or papillary	BAP1 tumour predisposition syndrome	<i>BAP1</i> ^a
Medulloblastoma	Fanconi anaemia	Positive chr breakage analysis (diepoxybutane test); <i>FANCA</i> ^a
Medulloblastoma, SHH-activated	ELP1-medulloblastoma syndrome	Heterozygous <i>ELP1</i> ; ^h <i>ELP1</i> ; ^c MP

ANNUBP – Atypical neurofibromatosis neoplasm with uncertain biologic potential; MPNST – Malignant peripheral nerve sheath tumour; CMMRD – Constitutional mismatch repair deficiency; MP – Methylome profiling; ^{wt} wildtype; ^a mutation/variant; ^b combined whole-arm deletion; ^c loss of heterozygosity; ^d structural variant; ^e partial/complete deletion; ^f expression; ^g loss of expression; ^h alteration.

For more information refer to the 2021 WHO CNS5 Tumour Classification.²

Note 1 – Adequacy of specimen for molecular assessment (Non-core)

The 2021 WHO CNS5 Tumour Classification uses histology, immunohistochemistry and molecular parameters to define many tumour entities.² Procuring viable and adequate tumour tissue allows appropriate histological and molecular assessment. However, the requirements for an adequate specimen for molecular assessment are not always the same as those for histological assessment. For example, ischemic times are critical for the quality of nucleic acid in general; the sooner samples can be frozen or fixed, the better. If immediate freezing or immediate appropriate fixation is not possible, placement in a refrigerator may reduce the degradation of nucleic acids.

Crush or freezing artefacts may affect adequacy for histopathology, including IHC or in situ hybridisation (ISH) testing, but do not often affect adequacy for molecular assays. Samples embedded in optimal cutting temperature (OCT) compound for cryostat sectioning can be a good source, and an advantage of using such samples is that one can evaluate tumour cell quantity as well as quality by checking histological sections of each sample.

Formalin-fixed, paraffin-embedded (FFPE) tissue samples also often provide a valuable source of information for molecular assessment. FFPE samples, however, can sometimes be more difficult for molecular biology assays because of fixation issues (such as overfixation and decalcification) that often cause nucleic acid degradation, resulting in fragmented DNA and RNA transcripts. Nonetheless, many laboratories have optimised molecular assays for FFPE tissue, given its commonplace nature.

Histological examination of tissue specimens used for nucleic acid extraction and subsequent molecular testing is essential to assure that vital tumour tissue with sufficient neoplastic cell content is being analysed. In certain cases, microdissection of cellular tumour areas may be required to ensure sensitivity of molecular analysis.

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Note 2 – *ALK/ROS1/MET/NTRK* family alterations (Core)*

***ALK* fusion or rearrangement**

The *ALK* (Anaplastic Lymphoma Kinase) gene (2p23) belongs to the insulin receptor superfamily of receptor tyrosine kinases (RTKs) and activates multiple downstream signal transduction pathways, including MAPK/PI3K/AKT/mTOR and JAK/STAT pathways.⁷ In cancer, *ALK* can be activated by gene fusions or point mutations/variants. Among gliomas, *ALK* fusions are mostly restricted to Infant-type hemispheric glioma (IHG) that are characterised by RTK gene fusions involving *ALK*, *ROS1*, *NTRK* or *MET*.^{8,9}

ALK fusions have been reported in about a third (39/130) of IHGs.⁸ Thirteen different fusion partners have been observed.⁸ All *ALK* fusions contain the complete *ALK* kinase domain at the C-terminal end, while the N-terminal partners retain variable domains in the chimeric protein, although most N-terminal partners have a coiled-coil or dimerisation domain.¹⁰

ALK fusions can be detected by ISH using dual-label break apart probes, next generation sequencing (NGS)-based gene fusion panel sequencing or whole transcriptome sequencing. Fusions lead to increased *ALK* protein expression that can be detected by IHC, a common method used to identify *ALK*-activated tumours. In a number of jurisdictions, IHC assays have been approved as companion diagnostics to aid in the identification of patients eligible for treatment with *ALK* inhibitors like crizotinib.^{11,12}

The most common indications for *ALK* fusion testing in CNS tumour diagnostics include intracerebral metastases of NSCLC (non-small cell lung carcinoma) in adults and a differential diagnosis of IHG in children.¹³ Among non-glial tumours that occasionally involve the CNS, *ALK*-positive anaplastic large cell lymphomas are characterised by oncogenic *ALK* fusions. *ALK* fusions have also been noted in *ALK*-positive histiocytosis, which is a novel type of systemic histiocytic proliferative disorder occurring predominantly in young children, rarely showing exclusive involvement of the CNS.^{14,15}

In addition to being a diagnostic marker for IHGs, *ALK* fusions also appear to be prognostic in this tumour type, although additional investigation is needed. One study⁹ observed a higher 5-year overall survival rate in patients with *ALK* rearranged tumours (53.8%) as compared to *ROS* and *NTRK* altered tumours. *ALK* rearranged tumours with low grade histology were diagnosed at an older age and had better survival rates as compared to those of patients with *ALK* altered high grade gliomas.

The presence of *ALK* fusions also provides opportunity for targeted therapy using *ALK* inhibitors, and early experience has demonstrated responses.^{13,16} Interestingly, different *ALK* fusion positive tumours have varying sensitivity to *ALK* inhibitors.¹⁰

***ROS1* fusion or rearrangement**

The *ROS1* (ROS proto-oncogene 1) gene (6q22.1) closely resembles *ALK* both in sequence and structure. Both receptors signal via the RAS/MAPK as well as the JAK/STAT and PI3K/AKT/mTOR pathways.¹³ *ROS1* fusions arise through intrachromosomal 6q micro-deletions in IDH-wildtype glioblastomas, although they are very rare as indicated by only 3 of 520 tumours in the Cancer Genome Atlas cohort.¹³ *ROS1* fusions are also reported in IHG.^{8,9} In a series of 118 infants with IHG, 7 (4%) were diagnosed with a *ROS1* fusion.⁹ Clark et al⁸ showed *ROS1* fusion as a driving alteration in 9/130 cases.

Immunohistochemistry (IHC), ISH (break apart probes), and DNA- or RNA-based NGS (custom panels or commercially available panels) can be used to detect *ROS1* fusion. However, each of these methods has limitations. IHC can be used as a screening method, yet it may identify tumours with increased protein expression in the absence of a fusion. Therefore, in case of a positive *ROS1* immunostaining, confirmation with either ISH or NGS should be performed.¹⁷

ROS1 fusions, though rare, are a diagnostic marker for IHGs in the appropriate clinical and radiological setting and also provide options for targeted therapy. Furthermore, one study⁹ reported that patients with tumours that harboured *ROS1* alterations had a lower 5-year overall survival rate as compared to those with *ALK* alterations (25% versus 53.8%).

Reports on successful targeted treatment of CNS tumours in children with *ROS1*-positive fusions such as IHG are anecdotal, but responses have been reported.^{18,19}

***NTRK* family alterations**

The neurotrophic tyrosine receptor kinase (*NTRK*) genes include *NTRK1*, *NTRK2*, and *NTRK3* and encode tropomyosin receptor kinases (TRKs), a family of RTKs involved in the development and maturation of the central and peripheral nervous system.

Although mutations and alternative splicing occur, fusions are the most common alterations of *NTRK* in tumours. The most common alteration is a fusion between an *NTRK* gene and another N-terminal partner. All these aberrations result in the constitutive activation of the kinase due to loss of the extracellular domain.¹⁰

NTRK fusions are reported in about 4% of paediatric gliomas and most of these are high grade. They are most prevalent in IHG.^{8,9,20} Clark et al⁸ reported that the most commonly targeted genes in their series were in *NTRK1/2/3*. *NTRK2* was found with numerous novel partners) but was largely seen in other glioma subtypes (e.g., H3K27M in midline regions), suggesting an important difference in *NTRK2* compared to *NTRK1/3* fusion-positive cases.

Among adult CNS tumours, NTRK fusions have been reported at a frequency of 1-2% in glioblastoma, IDH-wildtype.²¹ They have also been described in approximately 2% of pilocytic astrocytomas (PAs)²² and rarely in diffuse low grade gliomas, MAPK pathway altered.²³

NTRK fusion genes in CNS tumours can be assessed by ISH, reverse transcriptase-polymerase chain reaction (RT-PCR) or RNA-based sequencing (either panel based or whole transcriptome). Endogenous and physiological NTRK expression renders assessment by IHC in the nervous system challenging and thus molecular techniques are recommended.²⁴

NTRK fusions are diagnostic markers of IHGs and also appear to carry prognostic significance. In one study,⁹ it was reported that NTRK fusion-positive tumours had an intermediate prognosis as compared to *ALK* and *ROS* altered tumours in that the 5-year overall survival rate was 42.9%.

NTRK fusion provides an opportunity for targeted therapy with specific small molecule inhibitors. Larotrectinib and entrectinib have been conditionally approved by both the European Medicines Agency and the US Food and Drug Administration for NTRK fusion-positive cancers. Additional data regarding the efficacy of NTRK inhibitors in adult and paediatric CNS tumour patients are needed.^{10,13}

MET alterations

The *MET* oncogene (7q31.2) encodes hepatocyte growth factor/HGF, an RTK that plays a pivotal role in differentiation, cell proliferation, angiogenesis, migration, invasion, genomic stability and resistance to therapy.¹³ Dysregulation of *MET* signalling and activation of downstream pathways (RAS/MAPK, PI3K/AKT, and STAT pathways) can be caused by various mechanisms, including *MET* amplification, point mutation, fusion and *MET* exon skipping alteration.^{13,25,26}

Amplifications of *MET* have been reported in 0.7-6.2 % of IDH-wildtype glioblastomas and 5.2-17 % of IDH-mutant astrocytomas. They have also been identified in 20% of diffuse midline gliomas H3 K27-altered, in 10% of diffuse hemispheric gliomas H3 G34-mutant, and in gliomas arising after irradiation.^{27,28}

A variety of *MET* fusions have been detected in CNS tumours. *MET* fusions with variable N -terminal fusion partners have been described mainly in diffuse paediatric-type high grade gliomas, H3-wildtype and IDH-wildtype, IHG and in 3% of IDH-wildtype glioblastoma.^{8-10,13,29} Rarely, *MET* alterations have been reported in diffuse low grade gliomas, MAPK pathway-altered.²³

MET fusions in adult patients have been reported in a small subset of IDH-mutant astrocytomas (mainly in high grade and recurrent tumours) and in IDH wild type glioblastomas.^{10,30,31}

Exon skipping alterations of *MET* exon 14 and exon 7–8 have not been analysed to any significant extent in CNS tumours. However, few studies have reported these alterations in 6% to 8% of high grade gliomas.^{13,31} Similarly, the frequency of *MET* sequence variants in CNS tumours has not been precisely defined.¹³

There is no established and validated diagnostic test to assess *MET* alterations in CNS tumours by IHC. Specific *MET* protein antibodies have been tested to detect *MET* amplifications in gliomas, but standardised methods do not exist. Furthermore, the usefulness of *MET* IHC to detect *MET* fusions and exon skipping alterations is unclear. Hence, the preferred method is molecular detection of *MET* alterations.

Due to the frequent occurrence of multiple *MET* alterations simultaneously (e.g., amplifications, fusions with various breakpoints, exon skipping etc.), a hybrid capture based (targeted) DNA/RNA sequencing approach is recommended.^{13,26}

The detection of a *MET* fusion can aid in establishing the diagnosis of IHGs and also provides options for targeted therapy. The effectiveness of *MET* inhibitors in CNS tumours have been investigated in several phase I and II studies, but the effectiveness in biomarker-stratified cohorts with *MET* alterations has largely not been assessed.³²

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 3 – *ATRX* alterations (Core)*

The diagnosis of an IDH-mutant astrocytoma (CNS WHO grade 2, 3 or 4) is supported by the presence of *TP53* expression or alteration (mutation or deletion), in addition to loss of expression or alteration of the *ATRX* (α -thalassemia/mental retardation syndrome X-linked) gene (Xq21.1).^{33,34} Evaluation for these two markers is also commonly used to rule out the possibility of an oligodendroglioma, IDH-mutant and 1p/19q-codeleted.

Determination of *ATRX* loss of nuclear expression/mutations can be achieved in a number of ways, with a practical and cost-effective manner being IHC. The loss of nuclear *ATRX* immunostaining in neoplastic cells, with its maintained expression in non-neoplastic cells, such as endothelial cells or non-neoplastic glia, is strongly associated with *ATRX* genetic alterations and can be reliably used as a surrogate marker.³⁵ Mosaic staining patterns have also been reported, but these are not always associated with *ATRX* sequence alterations.³⁶ In combination with IHC for IDH1 R132H mutant protein and p53, *ATRX* IHC provides definitive results in the majority of cases, with the added benefit of preserving cytoarchitecture for microscopic examination.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 4 – *BCOR* internal tandem duplication (Core)*

Demonstration of a *BCOR* (BCL-6 Corepressor) internal tandem duplication (ITD) is required for the diagnosis of CNS tumour with *BCOR* ITD, introduced as a new embryonal tumour type in the WHO CNS5 Tumour Classification.²

This alteration consists of a solitary heterozygous *BCOR* ITD in exon 15 of the *BCOR* gene (Xp11.4). As its name implies, *BCOR* acts as an interacting corepressor of BCL-6, with an epigenetic regulator function, taking part in the polycomb repressive complex 1 (PRC1).³⁷ This alteration is also found in various other neoplasms such as small round cell sarcomas of soft tissues, clear cell sarcomas of the kidney and primitive myxoid mesenchymal tumour of infancy.³⁸

In routine neuropathological practice, RT-PCR or RNA sequencing by NGS could be used to detect *BCOR* ITDs, but strong and diffuse nuclear immunoexpression of *BCOR* is also a highly reliable and practical surrogate for

the presence of this alteration.³⁹ Furthermore, DNA methylation profiling can be used to classify CNS tumours with *BCOR* ITD as such, based on their methylation ‘fingerprint’.⁴⁰

In similarity with soft tissue tumours, rare CNS tumours sharing the same DNA-methylation cluster as CNS tumours with *BCOR* ITD, present with an alternative *BCOR* alteration such as deletion of *BCOR*, sequence variation of the *BCOR* gene or an *EP300::BCOR(L1)* fusion.⁴⁰ These tumours show magnetic resonance imaging homologies with CNS-*BCOR* ITD, but are significantly distinct from their *BCOR* ITD counterparts in terms of age, location, progression-free survival, tumour growth pattern, and also immunopositivity for the *BCOR* protein.^{41,42} Indeed, such CNS *BCOR* tumours with alternative alterations express variable *BCOR* by IHC with a high proportion of cases which are immunonegative.^{39,43,44}

Immunohistochemistry (IHC) for SATB2 has been reported as a sensitive but non-specific immunohistochemical marker for tumours with *BCOR* ITD and for alternative *BCOR* alterations.³⁹

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 5 – *BRAF* alterations (Core)*

BRAF genetic alterations

The *BRAF* (V-Raf Murine Sarcoma Viral Oncogene Homolog B protooncogene; 7q34) p.V600E sequence variant in exon 15, which is the most common *BRAF* alteration, affects a large variety of CNS tumours. It has been reported in 96% of papillary craniopharyngiomas, 65-75% of pleomorphic xanthoastrocytomas with and without anaplasia, 25-60% of gangliogliomas, 20–25% of dysembryoplastic neuroepithelial tumours, and 7% of Pas.^{45,46} *BRAF* variants have also been detected in about one-half of epithelioid glioblastomas and in up to 25% of diffuse astrocytic gliomas in children and young adults.⁴⁷ The detection of a *BRAF* sequence variant has diagnostic implications in specific tumours such as pleomorphic xanthoastrocytomas, ganglioglioma, dysembryoplastic neuroepithelial tumour, or epithelioid glioblastoma. Moreover, the detection of the variant can help to distinguish a ganglioglioma from the cortical infiltration of a diffuse glioma.

Besides its diagnostic value, *BRAF* sequence variants may allow for targeted therapy against mutant *BRAF* p.V600 protein. In paediatric low grade gliomas, the *BRAF* p.V600E sequence variant has been linked to poor response to conventional cytotoxic therapy and poor prognosis.⁴⁸ In routine settings, *BRAF* p.V600E can be identified by IHC or by molecular approaches such as high-resolution melting analysis, pyrosequencing, allele-specific quantitative polymerase chain reaction (ASQ-PCR), NGS and Sanger sequencing.⁴⁹ Although Sanger sequencing is a well-established tool to detect *BRAF* p.V600E and other rarer *BRAF* variants, it has a detection threshold of 20% (of mutant alleles). In samples that contain a minority of mutant cells, molecular methods with much lower thresholds, such as ASQ-PCR, droplet-based digital PCR (ddPCR), or NGS, are more sensitive.

BRAF p.V600E immunohistochemistry

Immunohistochemistry (IHC) is a commonly used method to detect *BRAF* p.V600E mutant protein in FFPE tissue of CNS tumours. Monoclonal antibodies (such as clone VE1 and clone V600E) against *BRAF* p.V600E are commercially available. Clone VE1 is the most widely used and is sensitive and specific.⁵⁰ The concordance between IHC and detection of the *BRAF* p.V600E variant by molecular genetic techniques demonstrates variability between studies in different types of neoplasms, but the overall concordance is strong.⁵⁰ IHC plays a key role when FFPE material available is not sufficient for molecular genetic analysis and when low tumour cell content may lead to false-negative results.

***BRAF* rearrangement/duplication**

Circumscribed duplication of the *BRAF* locus is a common copy number variation (CNV) that occurs in Pas of the cerebellum, hypothalamus, or optic chiasm, but may occur in Pas from other sites as well.

The mitogen-activated protein kinase (MAPK) signalling pathway is a key signalling pathway in the development of PA. The major alterations leading to constitutive activation of MAPK in Pas are gene fusions and missense variants involving *BRAF*, in particular *BRAF* p.V600E.

Identification of the *KIAA1549::BRAF* fusion has been used as a diagnostic marker for Pas. It has also been observed in pilomyxoid astrocytoma, ganglioglioma and diffuse leptomeningeal glioneuronal tumour (DLGNT). *KIAA1549::BRAF* fusions, while all coding for a fusion protein that includes the activating *BRAF* kinase domain, can be derived from at least nine different fusion site combinations. This makes RT-PCR a difficult method to identify or exclude all variants of the fusion gene. ISH analysis or ddPCR, which demonstrates the tandem duplication at 7q34, is an indirect way to indicate the presence of a *KIAA1549::BRAF* fusion. However, *BRAF* copy number gains due to trisomy 7 or whole 7q gains are common in glioblastomas, IDH-wildtype, and should not be mistaken as circumscribed *BRAF* duplication or *BRAF* fusion.

Methods that can identify all types of *BRAF* and *RAF1* fusion variants in a single experiment include RNA sequencing by NGS and methylation profiling.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 6 – C19MC alterations (Core)*

Demonstration of C19MC (C19MC microRNA cluster) alteration is required for the diagnosis of the most frequent molecular subtype of embryonal tumour with multilayered rosettes (ETMR).⁵¹ This alteration consists of *C19MC* amplification or fusion, typically a focal high-level amplicon of chromosome 19q13.42 covering a large, poorly characterised microRNA cluster and the miR-371-373 locus, which map about 100 kb apart. The width and the level of gains at this locus, as assessed by array-comparative genomic hybridisation (array-CGH), are variable but always encompass the same miRNA cluster.

Even in the absence of multilayered rosettes, a CNS embryonal tumour with *C19MC* alteration is diagnosed as ETMR, *C19MC*-altered.² In routine neuropathological practice, FISH or chromogenic ISH, or high-resolution cytogenetic techniques (e.g., array-CGH, single nucleotide polymorphism (SNP) arrays, methylation arrays) can be used to detect amplification of the C19MC region.

A small subset of ETMRs carry *DICER1* sequence variants. ETMRs lacking *C19MC* and *DICER1* alterations are designated as ETMR, NEC (not elsewhere classified), and those that are not tested for these alterations or in which the test results are inconclusive as ETMR, NOS (not otherwise specified).²

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 7 – *CDKN2A/B* deletion (Core)*

Homozygous deletion of the *CDKN2A/B* (cyclin-dependent kinase inhibitor 2A/B; 9p21.3) genes are associated with higher grade diffuse gliomas and have been introduced in the WHO CNS5 Tumour Classification as a marker for CNS WHO grade 4 IDH-mutant astrocytomas.²

In addition, *CDKN2A/B* homozygous deletions have been shown to be a characteristic genetic feature in pleomorphic xanthoastrocytomas, occurring in up to 87% of cases in one series. In this situation, along with *BRAF* p.V600E variant, the *CDKN2A/B* homozygous deletions do not connote more aggressive behaviour.⁵²

In neuropathological practice, high-resolution cytogenetic and molecular techniques (e.g., array-CGH, SNP arrays, methylation arrays, NGS arrays with copy number plots) can be used to detect homozygous *CDKN2A/B* deletions.

The *CDKN2A* gene encodes the p16 protein, which can be detected using IHC. However, loss of p16 nuclear staining cannot be recommended as a substitute for assessing homozygous *CDKN2A* deletion. In contrast, IHC for MTAP (S-methyl-50-thioadenosinephosphorylase, a product of the *MTAP* gene which is located on chromosome 9p21 in close proximity to the *CDKN2A* and *CDKN2B* loci) was found to show an excellent correlation with *CDKN2A/B* status in one study.⁵³

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 8 – Whole chromosomal arm 1p/19q codeletion (Core)*

Whole-arm deletions of chromosome arms 1p and 19q together with *IDH1* (isocitrate dehydrogenase (NADP(+)) 1) or *IDH2* missense variants constitute the diagnostic criteria for oligodendroglioma, IDH-mutant and 1p/19q codeleted, CNS WHO grades 2 or 3.²

Of note, only whole-arm 1p/19q codeletion combined with the *IDH* missense variant is diagnostically relevant. Partial deletions on either chromosome arm may be found in other types of diffuse gliomas, including IDH-wildtype glioblastomas, and are neither diagnostic for IDH-mutant and 1p/19q-codeleted oligodendroglial tumours² nor associated with favourable patient outcome.⁵⁴ Moreover, detection of 1p/19q codeletion in the absence of *IDH* mutation is suspicious of the partial deletions encountered in rare cases of IDH-wildtype glioblastoma.

Various techniques are being used for the diagnostic assessment of 1p/19q codeletion. Commonly used methods include microsatellite analysis for loss of heterozygosity (LOH), FISH or chromogenic ISH (CISH), and multiplex ligation-dependent probe amplification (MLPA).

Fluorescence or chromogenic ISH can be applied to routine FFPE sections. However, analysis is often restricted to single loci on each chromosome arm, which may not reliably distinguish whole-arm losses from partial deletions. In addition, polysomies of chromosomes 1 or 19 may complicate diagnostic assessment and have been associated with less favourable outcomes.⁵⁵

Loss of heterozygosity (LOH) analysis and MLPA assess multiple loci along each chromosome arm and thereby reduce the risk of false-positive findings due to partial deletions. However, extraction of tumour

DNA (and for LOH ideally also of non-tumour DNA extracted from a blood or buccal swab sample) is required for these techniques.

Microarray-based approaches may also be used for diagnostic purposes, including DNA methylation bead arrays that allow for simultaneous detection of 1p/19q codeletion, *MGMT* promoter methylation, and glioma CpG island methylator phenotype (G-CIMP) status indicative of IDH mutation.⁵⁶

Panel-based NGS approaches have been used for 1p/19q detection and simultaneous sequence alteration analyses of *IDH1* and *IDH2*, as well as other alterations commonly associated with 1p/19q codeletion, such as *TERT* promoter and *CIC* variants. In addition, ddPCR approaches based on SNPs on 1p and 19q may be used.

The use of an antibody panel including H3K28me3 (H3K27me3), H3 p.K28M (H3 p.K27M) mutant protein, IDH1 p.R132H,⁵⁷ vimentin, and ATRX,⁵⁸ have been reported as greatly facilitating recognition of oligodendrogliomas, IDH-mutant and 1p/19q-codeleted. However, these immunohistochemical approaches are not sufficient to substitute for 1p/19q codeletion testing and hence establishing the diagnosis of IDH-mutant and 1p/19q-codeleted oligodendroglioma.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 9 – Whole chromosome 7 gain (combined with whole chromosome 10 loss) (Core)*

Based on robust evidence in the literature, the Consortium to Inform Molecular and Practical Approaches to CNS Tumour Taxonomy – Not Official WHO (cIMPACT-NOW) update 3 recommended in 2018 the use of combined chromosome 7 gain and chromosome 10 loss (+7/-10) as a molecular criterion sufficient for identifying a histologically lower grade appearing (grade 2 or 3) IDH-wildtype diffuse astrocytic glioma, as glioblastoma IDH-wildtype (CNS WHO grade 4), especially in the elderly.⁵⁹ This recommendation has been adopted by the WHO CNS5 Tumour Classification.²

Partial gains on chromosome 7 and partial losses on chromosome 10 exhibited a diagnostic and prognostic value similar to that of complete +7 (trisomy 7) or complete -10 (monosomy 10).⁶⁰ Methods to detect the +7/-10 marker include ISH, ddPCR, MLPA, NGS, and array-based techniques including those used for methylome profiling.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 10 – *CIC* alterations (Core)*

The *CIC* (capicua transcriptional repressor) gene (19q13.2), or rarely *ATNX1/ATNX1L1* (ataxin-1; 6p22.3) gene fusion, is currently considered a requirement for the diagnosis of *CIC*-rearranged sarcoma, regardless of whether it arises within the CNS, soft tissue, bone, or viscera. Although originally considered ‘Ewing-like’ or part of the ‘Ewing sarcoma family’ of related undifferentiated round cell sarcomas, it has since been distinguished as a unique tumour type with a significantly worse prognosis than that of Ewing sarcoma.⁶¹

Potentially useful surrogate immunostains include CD99 (less extensive than Ewing sarcoma), WT1, ETV4, calretinin, MYC, NUT (for *CIC::NUTM1* fusion cases), and DUX4 (for *CIC::DUX4* fusion cases).⁶²⁻⁶⁷ However, a definitive diagnosis requires molecular confirmation to detect gene fusion via techniques such as ISH, RT-PCR, NGS (RNA or DNA), and anchored multiplex PCR. Methylation profiling may also be diagnostically useful, given that these tumours have a unique cluster that is distinct from other tumour types in the differential diagnosis.

CIC-rearranged and *ATNX1*-rearranged sarcomas of the CNS cluster together with their soft tissue counterparts, suggesting that they are likely the same tumour type despite the differing frequencies of fusion partners.^{66,68} Nevertheless, additional cases need to be studied to confirm this initial impression.

CIC gene alterations are also common in IDH-mutant and 1p/19q-codeleted oligodendrogliomas, although they typically consist in sequence variants in that tumour type.⁶⁹⁻⁷¹

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 11 – *DICER1* alterations (Core)*

The *DICER1* (dicer 1, ribonuclease III; 14q32.13) gene encodes the Dicer protein - a ribonuclease (RNase) III endoribonuclease that assists in producing microRNA, which regulates gene expression at the posttranscriptional level. Most sequence variants in this gene lead to an abnormally short DICER protein, which is likely unable to produce miRNA. Without regulation by miRNA, genes may be expressed abnormally, causing cells to grow and divide uncontrollably, leading to tumour formation.

A heterozygous *DICER1* germline variant with low penetrance causes DICER1 syndrome, a rare autosomal dominant familial tumour predisposition disorder.² Individuals with such a variant have an increased risk of developing many types of tumours, followed by secondary somatic missense variants in *DICER1* within five hotspot codons in the RNase IIIb domain.²

Primary intracranial *DICER1*-altered lesions include pineoblastoma, pituitary blastoma, primary intracranial sarcoma, and ETMRs lacking C19MC alteration. These tumours can have overlapping histological features, with primitive mesenchymal differentiation, rhabdomyoblastic and chondroid features, as well as spindle cell patterns.

Identification of a somatic *DICER1* pathogenic variant in tumour tissue may suggest the presence of a germline *DICER1* pathogenic variant.² Identifying a heterozygous germline pathogenic variant in *DICER1* establishes the diagnosis of *DICER1* syndrome. Molecular genetic testing approaches include gene-targeted and more comprehensive genomic testing, such as exome and genome sequencing.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 12 – *EGFR* alterations (Core)*

The *EGFR* (epidermal growth factor receptor; 7p12) gene is the most commonly amplified proto-oncogene in gliomas.⁷² *EGFR* amplification is detectable in approximately 40% of IDH-wildtype glioblastomas (CNS WHO grade 4) and is particularly common in tumours from adult patients with the classic or RTK type 2 molecular subtype of glioblastoma.^{27,73} Other CNS tumours that may carry *EGFR* amplifications include subsets of paediatric-type high grade glioma, IDH-wildtype and H3-wildtype, and rare instances of diffuse midline glioma, H3 K27-altered.

EGFR amplification is commonly associated with missense variants and genetic rearrangements, the most common of which, *EGFRvIII*, being detectable in about 50% of *EGFR*-amplified glioblastomas.^{74,75} *EGFRvIII* carries a unique peptide encoded by the fusion site of exons 1 and 8 that has served as a tumour-specific epitope for anti-*EGFRvIII* immunotherapy.⁷⁶

In adult-type diffuse gliomas, *EGFR* amplification (+/- *EGFRvIII*) is virtually restricted to glioblastoma, IDH-wildtype. The cIMPACT-NOW update 3 recommended in 2018 the use of *EGFR* amplification as a molecular criterion sufficient for identifying a histologically lower grade appearing (grade 2 or 3) IDH-wildtype diffuse astrocytic glioma as glioblastoma, IDH-wildtype (CNS WHO grade 4)⁵⁹. This recommendation has been adopted by the WHO CNS5 Tumour Classification.

Detection of *EGFR* amplification or *EGFRvIII* positivity also may be clinically relevant as a predictive marker of response to molecularly-guided therapies targeting *EGFR* and/or *EGFRvIII*.^{77,78}

EGFR amplification is usually seen in the majority of neoplastic cells in a given tumour and can be readily detected by ISH on routine FFPE tissue sections, although amplification levels may be heterogeneous from cell to cell. Targeted molecular techniques based on extracted tumour DNA, such as RT-PCR and MLPA, are also suitable for diagnostic detection of *EGFR* amplification. Microarray-based genomic or epigenetic analyses, as well as NGS approaches, are increasingly being used.⁷⁹

Gene amplification (defined by a circumscribed high-level copy number gain of the *EGFR* gene at 7p12) needs to be distinguished from low-level copy number gains of chromosome 7 caused by numerical chromosomal abnormalities, in particular trisomy 7, which is a frequent alteration in IDH-wildtype glioblastomas³⁴ (see also **Note 9 Whole Chromosome 7 gain (combined with whole chromosome 10 loss)**). To date, there is no evidence that different levels of *EGFR* gene amplification (e.g., increases in copy number of 10-fold versus 100-fold) have distinct diagnostic or prognostic impact.

Detection of *EGFRvIII* in *EGFR*-amplified glioblastomas can also be performed at the DNA level, e.g., by MLPA, microarray-based techniques and NGS. However, detection at the mRNA or protein level using RT-PCR or IHC with *EGFRvIII*-specific antibodies appears to be more sensitive.⁷⁴ This is due to the fact that *EGFRvIII* positivity usually shows regional heterogeneity and sometimes affects only a minor subset of the tumour cells.⁷⁴ Thus, representative sampling of tumour tissue is an important issue to avoid false-negative testing for *EGFRvIII*. Unfortunately, precise cut-off values for the distinction between high- and low-level copy number gains have not been defined and may need to be adjusted for each testing method.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 13 – *FET* alterations (Core)*

Fusions between members of the *FET* (nearly always *EWSR1*, but rarely *FUS*) and *CREB* (*CREB1*, *CREM* or *ATF1*) gene families help define a novel CNS tumour type referred to as intracranial mesenchymal tumour, *FET::CREB* fusion-positive.^{80,81} Given the histopathologic and genetic overlap with another rare soft tissue tumour type, prior cases have often been diagnosed as angiomatoid fibrous histiocytoma;⁸² however, recent methylation profiling studies suggest that these are likely two different entities.⁸¹

An immunohistochemical profile with combined EMA, CD99, and desmin should raise suspicion for this tumour type, but is not entirely specific.^{80,82} As such, confirmation of a *FET::CREB* fusion should be attained using various methodologies, including ISH, RT-PCR, NGS (RNA or DNA), and anchored multiplex PCR. Methylation profiling studies suggest that there may be two distinct epigenetic subtypes with differing clinicopathologic and prognostic associations.⁸¹ However, further studies are needed to confirm these findings in larger cohorts.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 14 – *FGFR* family alterations (Core)*

The *FGFR* (fibroblast growth factor receptor; 8p11.23) family comprises four highly conserved transmembrane tyrosine kinase receptors (*FGFR* 1-4) and one kinase-lacking core receptor (*FGFR* 5), which trigger downstream signalling pathways implicated in tumorigenesis, including the mitogen activated protein kinase (MAPK) pathway and the phosphoinositide-3-kinase (PI3K)/Akt pathways. *FGFR* plays key roles in CNS development, and in the context of neoplastic transformation it modulates tumour cell migration, differentiation, proliferation, and survival as well as angiogenesis.

FGFR gene alterations involve a broad spectrum of mutational types, such as hotspot point variants, fusions, internal domain duplication (ITD).⁸³ They are most commonly found in low grade glial or glioneuronal tumours, and less frequently in high grade gliomas. *FGFR1* hotspot variants are found across multiple tumour types, such as in PA, rosette forming glioneuronal tumour, dysembryoplastic neuroepithelial tumour, occasionally ganglioglioma and the rare diffuse low grade glioma, MAPK pathway-altered.⁸³ These hotspot variants have also been described in H3 K27M-altered diffuse midline gliomas and in diffuse gliomas in children and adults.

FGFR1::TACC1 fusions are common in extraventricular neurocytoma. *FGFR2::CTNNA3* fusions are characteristic of polymorphous low grade neuroepithelial tumour of the young.⁸³ In IDH-wildtype glioblastomas, *FGFR3::TACC3* fusions are rare events but are associated with distinct morphologic features (e.g., dense calcification, ‘chicken-wire’ capillaries, and bland oligodendrocyte-like cytology) and a better prognosis;⁸⁴ they are also mutually exclusive with *EGFR* amplifications.⁸³

Histologically, many tumours with *FGFR* alterations show neurocytic or oligodendroglioma like histological features.⁸³

There are no single useful histological or immunohistochemical surrogate markers to detect *FGFR* alterations. Therefore, the diagnostic approach usually requires a combination of methylome profiling, to narrow down or determine the tumour type (methylation class), and NGS to confirm DNA sequence variants or fusions (e.g., by DNA or RNA NGS, respectively).

FGFR alterations are clinically relevant, not only because of their diagnostic implications, but also because they may represent targets for cancer therapies,^{85,86} although evidence of efficacy in CNS tumours needs further evaluation.¹³

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 15 – *FOXR2* alterations (Core)*

Fusions accompanied by high levels of expression of the *FOXR2* (forkhead box R2; Xp11.21) gene in a CNS neoplasm with primitive neuroectodermal morphologic features are diagnostic of CNS neuroblastoma, *FOXR2*-activated.

FOXR2 fusions and resulting overexpression are best demonstrated using RNA sequencing techniques. The alterations are most commonly intragenic duplication events involving the *FOXR2* gene, or less commonly, intergenic translocations with non-recurrent partner genes.⁸⁷ Where RNA sequencing is not available, methylome profiling may be used to classify CNS neuroblastoma, *FOXR2*-activated. Surrogate immunohistochemical profiles combined with copy number alterations have also been proposed as sensitive and specific surrogate markers for CNS neuroblastoma, *FOXR2*-activated, namely: OLIG2, synaptophysin and SOX10 immunopositivity; vimentin negativity; and 1q gain.⁸⁸

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 16 – Histone H3 variants and loss of H3 p.K28me3 (K27me3) (Core)*

Various molecular methods can be used to detect the H3 p.K28M (K27M) variant, including pyrosequencing, TaqMan PCR, ddPCR, Sanger sequencing, and NGS. A similar array of methods can be used for H3.3 p.G35 (G34) variants; however, due to the GC rich nature of this region, targeted methods can be more difficult to design. For detection of both variants using targeted methods (and alignment of non-targeted methods), consideration needs to be given to the high degree of homology among the H3 genes (human H3 variants include H3.3, H3.1, H3.2, CENP-A, H3t, H3.X and H3.Y) and the number of genes encoding each protein (H3.3 is encoded by two genes, *H3F3A* and *H3F3B*, while H3.1 and H3.2 are each encoded by multiple genes found within gene clusters). The exact gene being tested, and the method used should be provided in the report. In addition, variant-specific antibodies are available that can reliably be used on FFPE tissue to detect H3 p.K28M (K27M), H3 p.G35R (G34R) and H3 p.G35V (G34V) variants.

Histone H3 p.K28M (K27M) variant (sequencing) and expression (immunohistochemistry)

Recurrent sequence variants in *H3-3A* (H3.3), *H3C2/3/11* (H3.1) are characteristic of diffuse midline gliomas, H3K27-altered. Very rarely *H3C14* (H3.2), with lysine 28 (27) substituted for methionine (H3 p.K28M (K27M)) or isoleucine (H2 p.K27I) also indicate diffuse midline glioma, H3 K27-altered.

These tumours can arise across a broad spectrum of ages and midline locations, including older adults, but are most frequently encountered in the paediatric age group in the pons. In teenagers and young adults,

non-pontine locations are more frequent, including the spinal cord, thalamus and cerebellum.⁸⁹ These tumours overall have a poor prognosis (median survival approximately 12 months) with older age at presentation and receipt of radiotherapy associated with modestly better survival.

The H3 p.K27M variant can also be found in diffuse astrocytomas without classic high grade features that generally behave more aggressively than their wild type counterparts. In occasional cases, this variant has been found in other tumour types, including ganglioglioma, PA and ependymoma. The outcome for patients with circumscribed low grade gliomas with H3 p.K27M variants is worse than their wildtype counterparts. Data on a small number of cases shows no outcome difference between PFA ependymomas with and without the H3 K27M variant, however.⁹⁰⁻⁹²

Testing for this alteration should be considered, in patients with midline diffuse gliomas. These alterations can be identified by sequencing or a variant-specific antibody. Detection of the variant by either IHC or sequencing is required for the diagnosis of the H3 p.K27M mutant subtypes of diffuse midline glioma, H3 K27-altered. Lack of H3 K27me3 is not a specific marker for H3 p.K27M.

Immunohistochemistry (IHC) with an antibody against the N-terminus of the mutant protein is highly sensitive and specific for detection of the H3 p.K28M (K27M) protein from either H3.3 or H3.1. In practice, the antibody can produce a fair amount of background cytoplasmic staining in non-tumour cells and only diffuse strong nuclear staining in most (or all) tumour cells should be considered positive. Further, poorly fixed tissue or tissue from post-mortem or older blocks may be false negative. If equivocal, a molecular method should be considered as the standard of care.

Histone H3 p.G35R (G34R) or p.G35V (G34V) variants (sequencing) and expression (immunohistochemistry)

Recurrent variants in *H3-3A* (H3.3) with glycine 35 substituted for arginine (H3 p.G35R) or infrequently valine (H3 p.G35V) are found most commonly in hemispheric high grade gliomas of the adolescent and young adult population.² The H3 p.G35R variant is found in approximately 15-20% of hemispheric high grade glioma cases in the paediatric age group.⁹³ Testing for this alteration should be considered, in hemispheric, IDH-wildtype, high grade gliomas, particularly if ATRX is lost and p53 is diffusely immunopositive. These alterations can be identified by sequencing or variant-specific antibodies against H3.3 p.G35R or H3.3 p.G35V.

In practice, the antibody works well for immunohistochemistry (IHC) on FFPE tissue with specific nuclear staining but does not stain all tumour cells; as a result, sensitivity may be an issue. If IHC results are equivocal or if suspicion for an H3 p.G35R/V variant is high, a molecular method should be considered as the standard of care.

Loss of H3 p.K28me3 (H3 K27me3) expression (immunohistochemistry)

The presence of the H3 p.K28M (K27M) mutant protein is associated with a fairly widespread (and thus detectable on Western blot or IHC) loss of the repressive trimethyl (me3) mark on H3 lysine 28 (H3 p.K28me3), often written as H3K27me3 when referring to the protein or antibody. Tumour cells harbouring the H3 p.K28M variant (either H3.1 or H3.3 p.K28M) will typically show loss of nuclear expression of H3K27me3 on IHC with retention of staining in entrapped non-neoplastic cells, e.g., endothelial cells (similar to the pattern seen with ATRX or INI1). However, it should be noted that while loss of H3K27me3 is sensitive for detection of H3 p.K27M variant tumours, it is not specific.

Other tumours, notably malignant peripheral nerve sheath tumours and some posterior fossa ependymomas,⁹⁴ will also show loss of H3 K27me3. In fact, in posterior fossa ependymomas this lack of immunoreactivity is considered a robust biomarker for posterior fossa group A (PFA) tumours.⁹⁴⁻⁹⁶

Similarly, in some H3-wildtype cases, partial loss may be seen. Thus, while helpful for confirmation when combined with an H3 p.K27M stain, loss of H3K27me3 staining by itself should be considered a non-specific surrogate marker for identifying H3 p.K27M-mutant diffuse midline gliomas.

In non-H3 pK27M-mutant subtypes of diffuse midline glioma, H3K27me3 is typically lost in combination with either EZHIP expression or an *EGFR* gene alteration.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 17 – IDH alterations (Core)*

IDH (isocitrate dehydrogenase) is an enzyme that exists in five isoforms, each of which catalyses the reaction of isocitrate to α -ketoglutarate. Hotspot missense variants in *IDH1* or *IDH2* are diagnostic markers for astrocytomas, IDH-mutant, CNS WHO grades 2-4 and oligodendroglioma, IDH-mutant and 1p/19q-codeleted, CNS WHO grade 2 or 3.²

The mutant IDH1 and IDH2 proteins lead to the production of the oncometabolite 2-hydroxyglutarate, which inhibits the function of numerous α -ketoglutarate-dependent enzymes. Inhibition of the family of histone demethylases and the ten-eleven translocation (TET) family of 5-methylcytosine hydroxylases has profound effects on the epigenetic status of mutated cells and leads to G-CIMP.

IDH2 variants are much less frequent than *IDH1* variants in diffuse gliomas, but are enriched in IDH-mutant and 1p/19q-codeleted oligodendrogliomas and in infratentorial IDH-mutant astrocytomas.⁹⁷

A monoclonal antibody has been developed to the IDH1 p.R132H protein, that allows for the detection of the most common type of IDH variant by IHC. The ability of the antibody to detect individual IDH R132H-mutant cells within a normal background (e.g., in the infiltration zone of an IDH-mutant diffuse glioma), makes this method more sensitive than Sanger sequencing for identifying p.R132H-mutant gliomas.⁹⁸ However, *IDH2* variants and other less common *IDH1* variants cannot be detected using IHC with this antibody, and in the proper clinical setting, it may be necessary to test for other *IDH1* and *IDH2* variants by sequencing analysis. The WHO CNS5 Classification recommends that sequencing may not be warranted in the setting of a negative p.R132H immunostain in glioblastomas arising in patients older than 55 years due to the rarity of non-R132H *IDH1* and *IDH2* variants in patients in this age group.⁹⁹ By contrast, all diffusely infiltrating gliomas with CNS WHO grade 2 and 3 histology that lack IDH1 p.R132H positivity by IHC should be assessed for less common *IDH1* or *IDH2* variants by sequencing or other appropriate methods.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 18 – MAPK pathway alterations (Core)*

The MAPK (mitogen-activated protein kinase) signalling pathway is a complex cellular signalling system involved in regulating a wide variety of cellular processes, including cell growth, differentiation, apoptosis, and response to stress. The pathway translates a diverse range of extracellular stimuli - including growth factors, cytokines, and environmental stressors - into cellular responses.

Different mechanisms can lead to dysregulation of the MAPK pathway in cancer. One common mechanism is the activation of growth factor receptors, which can stimulate MAPK pathway activation through various downstream signalling molecules. Genetic alterations in the MAPK pathway genes, such as *BRAF*, *NRAS*, *KRAS* and *NF1*, can also lead to hyperactivation and contribute to cancer development.

The MAPK pathway is frequently dysregulated in gliomas,² and this dysregulation is associated with increased proliferation and reduced apoptosis, leading to tumour growth and progression. The activation of the MAPK pathway is often driven by alterations in genes encoding the BRAF protein or the NF1 protein. These alterations lead to constitutive activation of the pathway, which promotes cell proliferation and survival and contributes to the development and progression of gliomas.

The importance of the MAPK pathway in cancer has led to the development of targeted therapies that inhibit its activity. Drugs inhibiting BRAF and/or MEK signalling, two critical components of the pathway, have been approved for treating melanoma and other cancers, including gliomas. However, resistance to these drugs can develop, highlighting the need for continued research into the complex mechanisms that regulate MAPK pathway activity in CNS tumours.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 19 – Methylome profiling (Core)*

The categorisation of CNS tumours based on their genome-wide methylome profiles greatly aids their precise classification,¹⁰⁰ often in combination with the DNA copy number profile derived from the same array.¹⁰¹ Methylome profiling can:

1. establish a methylation class (often a surrogate for a diagnosis) for most CNS tumour types including histologically ambiguous CNS tumours of both adult and paediatric patients;
2. subclassify or risk stratify established tumour types, such as ependymomas, medulloblastomas, or meningiomas; and
3. provide information on *MGMT* promoter methylation status.

Methylome profiling can also be useful as a diagnostic tool for very small biopsies. CNS tumours classified on the basis of their methylome profile generally behave clinically more homogeneously than those classified by histology alone.

The determination to perform methylation profiling depends on (i) local availability or access to supra-regional centres, (ii) availability of funding in the respective healthcare system, (iii) ability/knowledge to integrate data by a neuropathologist, as well as (iv) clinical need and relevance to therapy (e.g., risk prognostication of meningiomas).

The technology used for methylome-based tumour classification is currently mainly based on hybridisation of bead chip arrays from a single supplier. However, other approaches, such as long-read sequencing have been established.¹⁰² The processing of the methylation data requires a classification tool, and currently the gold standard for the diagnostic implementation is the DKFZ/Heidelberg classifier for CNS tumours (www.molecularneuropathology.org),¹⁰⁰ although alternatives based on the same principle have been established to address accreditation issues or to refine classification.¹⁰³

The implementation and acceptance of this technology has substantially empowered the neuropathology community by enabling more accurate, reliable, and reproducible diagnoses. Thus, DNA methylome-based classification has been widely introduced as a diagnostic tool in the WHO CNS5 Tumour Classification.¹⁰⁴ In fact, unequivocal classification of certain tumour types, such as high grade astrocytoma with piloid features and paediatric-type high grade diffuse gliomas IDH-wildtype and H3-wildtype, require the demonstration of a tumour type-specific methylome profile.¹⁰⁴

The use of the DNA methylome classifier requires caution and awareness of potential pitfalls.¹⁰³ Technical and operational risks include recognition of sample mix-up, low DNA amount and poor quality DNA. It is recommended to process reasonably distinct tumour entities on each chip (currently eight samples per chip), and results that cannot be reconciled with patient sex, histology, location, or clinical presentation may require repeat investigation. Interpretational pitfalls arise from incorrect classification results, due to low tumour content, e.g., admixture of CNS tissue, inflammatory cells, tumour recurrences/post-radiotherapy, and tumours arising in genetic tumour syndromes.

Generally, low grade glial and glioneuronal tumours can be difficult to classify with the current algorithms.²³ The DKFZ/Heidelberg classifier uses calibrated classifier scores to indicate likelihood of the assignment of a tumour to a distinct methylation class,¹⁰⁰ with classifier scores >0.9 indicating a significant match. Lower calibrated classifier scores need to be interpreted with caution and may not be reliable indicators of a certain diagnosis but can still provide useful guidance when integrated with results from orthogonal tests.^{100,103}

Copy number variations (CNV) are returned as part of the readout from the methylation array (or from long-read whole genome sequencing), and can complement the diagnosis, provide additional confidence in establishing a diagnosis when the methylome profile is returned with a low calibrated score,¹⁰¹ or form part of a prognostication algorithm, such as in meningiomas.¹⁰⁵ Therefore, CNVs (including specific gene deletions or amplifications) should also be included in the report if diagnostically relevant. Gene duplication and/or gene fusions can sometimes also be inferred from the plot but may need confirmation by other methods. Low amplitudes of CNVs may indicate low tumour cell content or clonal heterogeneity in the investigated tissue sample.

No specific formal recommendations exist currently for how methylome data should be reported. It has been suggested that pathology reports should contain information on:

1. estimated tumour cell content of the extracted DNA,
2. amount of DNA input,
3. estimated tumour cell fraction,
4. quality of bisulphite conversion,
5. CNS tumour classifier version(s) used,
6. highest scoring methylation category with the respective calibrated score(s), and
7. sub-classification with score(s) if applicable.⁶

In addition to the DNA copy number profile and assignment to distinct methylation families, classes and subclasses - the DKFZ/Heidelberg classifier provides the *MGMT* promoter methylation status based on a specific algorithm.¹⁰⁶ Generally, there is good concordance with other methods of targeted assessment of *MGMT* promoter methylation.^{107,108} However, there is currently no consensus as to which testing method best predicts response to alkylating agent chemotherapy.¹⁰⁹

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 20 – *MN1* alterations (Core)*

Demonstration of an *MN1* (meningioma 1; 22q12.1) gene alteration is required for the diagnosis of astroblastoma, *MN1*-altered; which is new tumour type introduced in the WHO CNS5 Tumour Classification, without an assigned CNS WHO grade.² These rare glial neoplasms have a strong female predominance and are characterised by relatively compact growth, predominantly perivascular tumour cell arrangement (astroblastic rosette) and perivascular fibrous stroma.

Fusions between *MN1* (22q12.1) and BEN domain containing 2 (*BEND2*; Xp22.13) or more rarely with *CXXC5* (*CXXC*-type zinc finger protein 5) are characteristic. *MN1* is a transcriptional coregulator important in development and is implicated in the pathogenesis of meningioma and acute myeloid leukemia.^{110,111} Astroblastomas, *MN1*-altered display a distinct DNA methylome profile. Tumours with astroblastoma-like histology and DNA methylome profile, often located in the spinal cord, have been identified which lack *MN1* fusions but feature *BEND2* fusions, in particular *EWSR1::BEND2* or *MAMLD1::BEND2*. These tumours likely represent a molecular subtype of astroblastoma, however, have not yet been considered in the WHO CNS5 classification.

Molecular studies are necessary to evaluate for characteristic *MN1* or *BEND2* fusions by break apart ISH, PCR, RNA or DNA sequencing.

Nuclear *MN1* immunoreactivity has been described in *MN1::BEND2* tumours but not in non-*MN1::BEND2* astroblastomas.¹¹² However, the specificity and sensitivity of this biomarker remains to be evaluated.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 21 – *MYB*, *MYBL1* alterations (Core)*

The *MYB* (v-myb avian myeloblastosis viral oncogene homolog; 6q23.3) and *MYBL1* (8q13.1) rearrangements, most commonly gene fusions of *MYB* or *MYBL1* with various partner genes, are diagnostic alterations in diffuse astrocytoma, *MYB/MYBL1*-altered and in angiocentric glioma, with the latter typically featuring *QKI::MYB* fusions.^{104,113} *MYB* gene amplification is rare.^{114,115}

Diagnostic detection of *MYB* or *MYBL1* fusions can be performed by targeted next generation fusion panel sequencing or by whole transcriptome sequencing. Alternatively, *MYB/MYBL1* alterations can be detected by using interphase ISH. DNA methylome analysis also identifies gliomas with *MYB* or *MYBL1* alterations but cannot distinguish between diffuse astrocytoma, *MYB/MYBL1*-altered versus angiocentric glioma.^{114,116}

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 22 – *MYC* gene family amplification (*MYC* and/or *MYCN*) (Core)*

The *MYC* (v-myc avian myelocytomatosis viral oncogene homolog; 8q24.21) protein has a fundamental role in cell proliferation, cell size, differentiation, stem cell self-renewal, and apoptosis. Its deregulation occurs in many cancers including a range of CNS tumours. The *MYC* transcription factor family also includes its paralogues *MYCN* and *MYCL*.¹¹⁷

MYC, *MYCN*, and *MYCL* amplifications are prognostically relevant in medulloblastomas.¹¹⁸ *MYC* and *MYCN* gene amplification and fusions are seen in the SHH group, and non-WNT/non-SHH, but almost never in WNT-activated medulloblastomas.^{118,119} Furthermore, *MYCN*-amplification is listed in the WHO CNS5 Tumour Classification as an essential diagnostic criterion for spinal ependymoma, *MYCN*-amplified.² Rarely, such aggressive spinal ependymomas may show amplification of *MYC*.¹²⁰

A commonly used laboratory method to detect *MYC* gene family amplifications is FISH or CISH. Other approaches include RT-PCR, NGS, MLPA, or array technologies.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 23 – *PDGFRA* alterations (Core)*

PDGFRA (platelet-derived growth factor receptor, alpha polypeptide; 4q12) gene alterations have been described in several CNS tumour types, including low grade and high grade gliomas as well as glioneuronal tumours. These alterations include gains/amplifications and/or DNA sequence variants of *PDGFRA*.

PDGFRA amplifications and/or sequence alterations are found in variable frequency in both paediatric-type and adult-type high grade gliomas including diffuse paediatric-type high grade gliomas, H3-wildtype and IDH-wildtype;¹²¹ diffuse midline gliomas, H3 K27-altered;¹²² diffuse hemispheric gliomas, H3 G34-mutant;¹²³ glioblastomas, IDH-wildtype;¹²⁴ and about a third of astrocytomas, IDH-mutant, CNS WHO grade 4.¹²⁵ In the right context, the *PDGFRA* p.K385L variant is diagnostic of myxoid glioneuronal tumour.¹²⁶⁻¹²⁸

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 24 – Pituitary hormones and transcription factors immunohistochemistry (Core)*

Standard IHC evaluation of pituitary neuroendocrine tumours/pituitary adenomas includes immunostaining for specific anterior pituitary hormones (prolactin, growth hormone, follicle stimulating hormone, luteinising hormone, thyroid stimulating hormone, alpha-subunit of glycoproteins, adrenocorticotrophic hormone (PRL, GH, FSH, LH, TSH, ASU, ACTH, respectively) and pituitary transcription factors (PIT1, TPIT, SF1).^{129,130} Other transcription factors including GATA3 and ER α may be useful in certain instances.¹²⁹

Immunohistochemistry (IHC) for these proteins, coupled with cytokeratin (AE1/AE3 or CAM5.2) staining, for presence or absence of rounded cytoplasmic inclusions known as fibrous bodies, allows classification of pituitary tumours/pituitary adenomas for prognosis and medical treatment purposes.

For diagnostic purposes, some advocate first screening with three antibodies (PIT1, SF1, and TPIT) and then using the other anterior pituitary hormone assays based on initial results.¹³¹⁻¹³³ Others utilise the full panel initially and may variably supplement the panel.¹³⁴ The proliferation-associated marker Ki-67 (MIB1) is used for evaluation of the proliferative potential of tumours.^{129,134} There appears to be little utility for p53 IHC, with rare exceptions such as corticotroph tumours/adenomas.^{129,135,136}

The WHO 5th edition CNS² and Endocrine¹³⁷ Classification systems note that: “Special tumour/adenoma subtypes that commonly show aggressive behaviour...include sparsely granulated somatotroph tumour/adenoma, lactotroph tumours/adenomas in men, Crooke cell tumour/adenoma and silent corticotroph tumour/adenoma, and immature PIT1-lineage adenoma (previously called ‘silent subtype 3 adenoma’).”

For tumours of the posterior pituitary gland (granular cell tumour of the sellar region, pituicytoma, spindle cell oncocytoma), nuclear staining for the transcription factor TTF1 is used as a diagnostic marker.¹³⁸

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 25 – *PRC2* inactivation (Core)*

PRC2 (polycomb Repressive Complex 2) is an epigenetic regulator that is frequently inactivated in malignant peripheral nerve sheath tumours (MPNSTs).

In MPNSTs, loss-of-function genetic alterations or epigenetic silencing of PRC2 components, such as EZH2 (Enhancer of Zeste Homolog 2) or SUZ12 (Suppressor of Zeste 12), results in reduced H3K27me3 levels at target gene promoters. Consequently, this dysregulation leads to the activation of genes promoting tumour progression.

H3K27me3 loss has been shown to be a relatively specific marker for MPNSTs and may be helpful in the differential diagnosis to distinguish MPNSTs from histologic mimics, especially when supportive Schwann cell markers are absent.¹³⁹

Components of the PRC2 complex, such as EZH2, represent therapeutic targets undergoing clinical trials and further research.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 26 – *PRKAR1A* inactivation (Core)*

PRKAR1A (protein kinase, cAMP-dependent, regulatory, type I, alpha; 17q24.2) encodes the regulatory subunit of cyclic AMP-dependent protein kinase A (PKA) and is associated with Carney complex, a syndrome characterised by an increased risk of several types of tumours, including malignant melanotic nerve sheath tumours. Malignant melanotic nerve sheath tumours demonstrate frequent loss of function alterations in *PRKAR1A*.¹⁴⁰ *PRKAR1A* alterations can be in the form of single base pair substitutions, deletions and insertions, or rearrangements.

Loss of PRKAR1A expression can also be detected using IHC.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 27 – *PRKCA* inactivation (Core)*

PRKCA (protein kinase C alpha; 17q24.2) encodes a protein kinase involved in cellular signalling pathways related to proliferation and differentiation. *PRKCA* alterations, including gene rearrangements, are diagnostic for papillary glioneuronal tumours,¹⁴¹ which can be challenging to classify and thus are an essential WHO criterium for these tumours.²

In addition, the hotspot *PRKCA* missense variant p.D463H is highly specific for chordoid gliomas and is considered a desirable diagnostic WHO criterium for these tumours.²

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 28 – *SHH* pathway alterations (Core)*

About 30% of all medulloblastomas are characterised by SHH (sonic hedgehog) pathway activation, caused by genetic alterations in *PTCH1*, *SUFU*, *SMO*, or other genes encoding components of the SHH signalling pathway.

In SHH-activated medulloblastomas the *TP53* status needs to be assessed for a precise diagnosis as this group encompasses two very different disease entities. *TP53*-wildtype SHH-activated medulloblastomas occur mostly in adolescents/adults and young children and are associated with a good prognosis if adequately treated. In contrast, *TP53*-mutant SHH-activated medulloblastomas typically occur in older children and have a dismal prognosis.

A substantial subset of the SHH-activated medulloblastomas has the desmoplastic/nodular (D/N) phenotype and a small minority concerns medulloblastomas with extensive nodularity. The large cell/anaplastic (LC/A) phenotype is relatively frequent in the group of *TP53*-mutant SHH-activated medulloblastomas.

SHH activation can reliably be assessed by immunohistochemical cytoplasmatic staining for the SHH target proteins GAB1 and p75NGFR. Furthermore, these medulloblastomas share expression of nuclear YAP1 with WNT-activated medulloblastomas but lack OTX2 expression as well as nuclear accumulation of β -catenin protein. DNA methylation and mRNA expression profiles can be used for detecting SHH-activated medulloblastomas as well. As germline alterations are relatively frequent in patients with SHH-activated medulloblastoma, this tumour requires genetic counselling.

The canonical inherited syndrome associated with *TP53*-wildtype SHH-activated medulloblastoma is naevoid basal cell carcinoma (Gorlin) syndrome, which is mostly due to inactivating germline alterations in *PTCH1* (encoding the receptor for the SHH protein), and more rarely due to a *SUFU* or *PTCH2* mutation. Germline alterations in *ELP1* and in *GPR161* have also been reported in SHH-activated medulloblastomas. More than half of the patients with a SHH-activated and *TP53*-mutant medulloblastomas have germline (rather than somatic) *TP53* alterations (Li-Fraumeni syndrome).

Widespread and strong immunohistochemical staining for p53 in a SHH-activated medulloblastomas strongly indicates a *TP53*-mutant tumour. Most of these tumours show cytological anaplasia, at least focally. Ideally, because of the important consequences for treatment decisions and possible germ line alterations, SHH-activated tumours should be sequenced for presence/absence of *TP53* alterations.^{2,142-145}

Non-WNT/non-SHH medulloblastomas express OTX2 but lack staining of tumour cells for YAP1, GAB1, p75NGFR and nuclear β -catenin. Also, non-WNT/non-SHH medulloblastomas are generally not associated with genetic tumour syndromes (only rare cases have been reported in individuals with a germline alteration in *CREBBP* (Rubinstein–Taybi syndrome) or in the DNA repair genes *PALB2* or *BRCA2*).¹⁴³

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 29 – *SMARC* family alterations (Core)*

Atypical teratoid/rhabdoid tumour (AT/RT) is defined as a CNS embryonal tumour that frequently (but not invariably) contains rhabdoid cells and demonstrates inactivation of the *SMARC* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin) genes *SMARCB1* (INI1) or *SMARCA4* (BRG1).

SMARCA4/BRG1 alterations

The *SMARCA4* gene (19p13.2) encodes the transcription activator BRG1, also known as adenosine triphosphate (ATP)-dependent chromatin remodeller SMARCA4. AT/RTs with *SMARCA4* loss are extremely rare. Loss of BRG1 expression (and retention of INI1 expression) in these tumours can be readily demonstrated by IHC. Associated genetic alterations of *SMARCA4*, whether copy number alterations or mutations, can be detected by a variety of array or sequencing methods.

SMARCB1/INI1/SNF5 alterations

Inactivation of the *SMARCB1* (*INI1*, *BAF47*, *SNF5*) gene (22q11.23) is present in almost all cases of AT/RT, resulting in nuclear loss of SMARCB1 protein which can be evaluated by IHC. Genetic aberrations of the *SMARCB1* locus may include homozygous or heterozygous deletions and a variety of coding sequence variants, leading to inactivation of both alleles. However, genetic testing is usually not required for making the diagnosis of AT/RT because IHC is highly sensitive.

Since SMARCB1 is a constitutively expressed protein, IHC staining for SMARCB1 in the nuclei of non-neoplastic cells (such as vascular and inflammatory cells) serves as an internal positive control. Some AT/RTs with nuclear loss of SMARCB1 exhibit cytoplasmic staining, possibly representing dysfunctional truncated protein.

In tumours with histological features of AT/RTs but without demonstration of SMARCB1 or SMARCA4 alterations, only a diagnosis of ‘CNS embryonal tumour with rhabdoid features, NEC’ can be made.

A variety of other tumour types that involve the nervous system may exhibit loss of nuclear SMARCB1, including cribriform neuroepithelial tumour, poorly differentiated chordoma, rhabdoid tumour of the sellar region, myxoid meningeal tumours, and sinonasal carcinoma.² The molecular and nosologic relationship of these tumours to AT/RT is unclear. Furthermore, complete or incomplete (reduced, mosaic) loss of SMARCB1 protein expression has been found in some cases of choroid plexus carcinoma, synovial sarcoma, epithelioid schwannoma, and schwannoma associated with schwannomatosis.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 30 – STAT6 immunohistochemistry and rearrangement (Core)*

In-frame *NAB2::STAT6* gene fusions result from chromosome 12q13 inversions and represent highly sensitive and specific signature alterations of meningeal solitary fibrous tumours (SFT) of grade 1, 2, or 3; these fusions are also characteristic of the analogous soft tissue/extracranial counterparts.

STAT6 (signal transducer and activator of transcription 6) staining of tumour cell nuclei is a highly reliable and practical surrogate for detecting this signature alteration, with nearly 100% sensitivity and specificity regardless of the fusion variant,^{146,147} and is listed as essential diagnostic criterion for SFT in the WHO CNS5 Tumour Classification.²

***NAB2::STAT6* gene fusion**

NAB2::STAT6 gene fusions are detectable using RT-PCR, ISH or various sequencing techniques, including NGS if designed appropriately.^{146,148} Over 40 fusion variants have been detected to date, with the most common meningeal SFT-associated fusions bringing together exon 6 of *NAB2* (NGFI-A-binding protein 2; 2q13.3) with exons 16, 17, or 18 of *STAT6* (roughly one-half of all cases).¹⁴⁸

STAT6 nuclear expression (immunohistochemistry)

The STAT6 protein is normally expressed in the cytoplasm of cells, whereas NAB2 is expressed in nuclei; however, the *NAB2::STAT6* fusions cause the STAT6 protein to translocate to the nucleus. Nearly all meningeal and extracranial SFTs display strong and extensive/diffuse nuclear positivity, whereas other diagnostic considerations, such as meningiomas, nerve sheath tumours, and various sarcomas, either lack expression or show only cytoplasmic staining. As such, the pathologist is cautioned against rendering a diagnosis of SFT in the absence of nuclear STAT6 immunoreactivity.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 31 – *TERT* promoter alterations (Core)*

The *TERT* (telomerase reverse transcriptase; 5p15.33) gene encodes a major component of the protein complex telomerase and contributes to maintaining telomere length. Sequence variants in the *TERT* promoter create new binding sites for erythroblast transformation specific transcription factors and subsequently increase expression and activity of telomerase.

TERT promoter variants are detectable in the majority of glioblastomas, IDH-wildtype and of oligodendrogliomas, IDH-mutant and 1p/19q-codeleted, but are typically absent in astrocytomas, IDH-mutant.^{149,150}

In 2018, cIMPACT-NOW update 3 recommended the use of *TERT* promoter alteration as a molecular criterion for identifying a histologically lower grade appearing (grade 2 or 3) IDH-wildtype, adult-type diffuse astrocytic glioma as glioblastoma, IDH-wildtype (CNS WHO grade 4).⁵⁹ This recommendation has been adopted by the WHO CNS5 Tumour Classification.² However, some authors have suggested using caution when assimilating IDH-wildtype, histologically grade 2 gliomas to ‘molecular glioblastomas’ in case of ‘isolated *TERT* promoter mutation’ (lacking *EGFR* amplification as well as combined gain of whole chromosome 7 and loss of whole chromosome 10).^{151,152} DNA methylome profiling and NGS may

substantiate the diagnosis in such cases by demonstrating methylome profiles and additional genetic alterations of IDH-wildtype glioblastoma.

About 20% of medulloblastomas carry *TERT* promoter alterations, and they are more common in adult patients and in the SHH-activated molecular type.¹⁴⁹ In meningiomas, *TERT* promoter alterations have been found in 6% of tumours where they represent a marker of poor prognosis and according to the WHO CNS5 Tumour Classification can be used to assign a CNS WHO grade 3.¹⁵³ About 50% of SFTs carry a *TERT* promoter alteration while other tumours of the CNS only uncommonly exhibit these alterations.¹⁴⁹

Two hotspot missense variants (abbreviated as C228T and C250T) represent the vast majority of *TERT* promoter alterations in CNS tumours. Other variants have been rarely detected in CNS tumours, such as C228A and C249T in gliomas.¹⁴⁹ *TERT* promoter variants can be detected by various molecular techniques with Sanger sequencing, NGS and RT-PCR being most commonly used.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 32 – *TP53* alterations (Core)*

Sequence variants in the *TP53* (tumour protein p53; 17p13.1) gene are found in a variety of cancers including >80% of IDH-mutant diffuse astrocytic gliomas.³³ *TP53* variants are less common in IDH-wildtype glioblastomas (23-28%) and are notably uncommon in oligodendrogliomas, IDH-mutant and 1p/19q-codeleted.

Detection of a *TP53* variant may be used to support the diagnosis of IDH-mutant astrocytoma. However, exclusion of 1p/19q codeletion and ATRX loss of expression is not sufficient to establish this diagnosis, as rare cases of usually high grade and/or recurrent oligodendroglioma, IDH-mutant and 1p/19q-codeleted may also feature *TP53* variants. Furthermore, *TP53* variants are important for classifying medulloblastomas, SHH pathway-activated and *TP53*-mutant. *TP53* alterations are common in some other types of brain tumours but are not used diagnostically as in the above situations.

Different DNA sequencing techniques may be used for detecting *TP53* variants, with NGS covering the entire coding sequence being most reliable, as sequence alterations tend to cluster in exons 5 to 8 but may also affect other exons. The vast majority of alterations are missense variants.

Immunohistochemistry (IHC) is a useful screening tool, given that most *TP53* missense variants result in increased p53 protein half-life that produces strong immunoreactivity in the majority of tumour cell nuclei (rather than scattered positivity and/or light nuclear staining). Strong p53 positivity in >10% of the tumour cell nuclei has been found to have a sensitivity of 77.4-78.8% and a specificity of 78.6-96.7% when compared to sequencing.^{154,155} Positive nuclear p53 staining correlates well with missense sequence variants with a sensitivity of 92% and a specificity of 79.4%, whereas only 33% of tumours with truncating *TP53* alterations show p53 positivity,¹⁵⁵ with such alterations typically leading to negative staining.¹⁵⁶

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 33 – TTF1 expression (IHC) (Core)*

The TTF1 (thyroid transcription factor 1; 14q13.3) protein, encoded by the homeobox gene *NKX2-1* (14q13.3),¹⁵⁷ is essential for organogenesis of lung, thyroid, and the ventral forebrain.

The expression of TTF1 in the human embryonic and adult brain appears to be restricted to the ventral forebrain and diencephalic origin structures. TTF1 is expressed by normal pituicytes of the neurohypophysis,¹⁵⁸ ependymal cells of the third ventricle and glial cells of the organum vasculosum of the lamina terminalis.¹⁵⁹

TTF1 expression in CNS tumours has been reported in posterior pituitary tumours including pituicytomas, spindle cell oncocytomas and granular cell tumours of the neurohypophysis, in ependymomas of the third ventricle, subependymal giant cell astrocytomas, and chordoid gliomas.¹⁵⁸⁻¹⁶⁵ In addition, glioblastomas with primitive neuronal components may express TTF1 in the embryonal, GFAP-negative tumour cell component depending which antibody clone is being utilised.¹⁶⁶

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 34 – WNT pathway alterations (Core)*

About 10% of all medulloblastomas are characterised by WNT pathway activation by a *CTNNB1* (catenin (cadherin-associated protein), beta 1) activating variants in exon 3 – or rarely – sequence variants in *APC* (adenomatous polyposis coli) or other genes encoding components of this pathway. These medulloblastomas cannot be identified as such based on haematoxylin-eosin stained sections alone. Most of them have classic morphology. The precise identification of these tumours is important because of their favourable prognosis in the paediatric age (<16 years), allowing for reduction of treatment intensity.

Immunohistochemically, WNT-activated medulloblastomas typically show YAP1 staining of tumour nuclei, nuclear expression of OTX2, and are negative for the SHH target proteins GAB1 and p75NGFR. Furthermore, in most WNT-activated medulloblastomas at least some tumour cell nuclei are positive for β -catenin protein, but discrimination from strong cytoplasmic staining of the tumour cells may be challenging. It has been recommended to use at least two of the following methods for reliable identification of WNT-activated medulloblastomas: IHC, sequencing of *CTNNB1* exon 3, methylome profiling, RNA profiling.

Rarely, WNT-activated medulloblastomas are diagnosed within the setting of constitutional mismatch repair deficiency syndrome or in individuals with germline *APC* alterations and a predisposition to colon cancer, but the vast majority of these medulloblastomas are sporadic.^{2,143,144,167}

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 35 – *YAP1* rearrangement (Core)*

In the WHO CNS5 Tumour Classification, supratentorial ependymoma, *YAP1* (yes-associated protein 1; 11q22.1) fusion-positive, has been introduced as a separate tumour type.² Supratentorial ependymomas with *YAP1* fusion are rare and mostly restricted to young children.¹⁶⁸

Fusions involving the *YAP1* gene can be detected by a variety of methods; however, an IHC approach is currently not available. Transcriptome sequencing can detect *YAP1* fused to several gene partners, such as *MAMLD1*.¹⁶⁸ Methods using RT-PCR or interphase ISH are alternatives.¹⁶⁸

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 36 – *ZFTA* rearrangement (Core)*

In the WHO CNS5 Tumour Classification, the supratentorial ependymomas formerly coined as *RELA* (v-rel avian reticuloendotheliosis viral oncogene homolog A; 11q13.1) fusion-positive are now listed as supratentorial ependymoma, *ZFTA* (zinc finger translocation associated; 11q13.1) fusion-positive (with *ZFTA* being the new designation for *C11orf95*; 11q13.1).² Approximately two-thirds of supratentorial ependymomas in children are *ZFTA* fusion-positive, with in the vast majority of these harbouring *RELA* as the fusion partner.^{2,168} Demonstration of a *ZFTA* fusion is required for their diagnosis in the WHO CNS5 Tumour Classification.²

These fusions can be identified by RNA sequencing, RT-PCR based techniques, or ISH; whole genome sequencing can also detect the fusion. Targeted RNA sequencing and RT-PCR design should take into consideration the complex nature of the fusion events generated by chromothripsis on chromosome 11.

In situ hybridisation (ISH) probes against either *ZFTA* or *RELA* may be used to detect chromosome 11 rearrangements.¹⁶⁹ *ZFTA* fusion-positive ependymomas with or without *RELA* represent the same tumour entity in the WHO CNS5 Tumour Classification. It is known that a broader spectrum of tumours than classic ependymomas exhibit *ZFTA* fusions without *RELA*.¹⁷⁰ Supratentorial ependymomas without *ZFTA* (and without *YAP1*) fusion also exist.¹⁷¹

L1CAM (cytoplasmic staining) and p65 (nuclear staining) in cases with *ZFTA::RELA* fusions, represent surrogate IHC markers for *ZFTA* fusion-positive tumours. Strong and diffuse L1CAM immunopositivity is a sensitive but not a specific surrogate marker as it can also be expressed by other tumour types. Nonetheless, L1CAM IHC is recommended for indicating that a supratentorial ependymoma likely belongs to the *ZFTA* fusion-positive category, when fusion testing is not possible or yields equivocal results.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 37 – Other immunohistochemistry findings (Core)*

A growing number of IHC tests represent core ICCR or essential WHO diagnostic criteria, including IHC for Brachyury, CD34, EZHIP, GFAP, S100, class III β -tubulin, neurofilament, synaptophysin, NeuN, OLIG2,

HuC/HuD, non-phosphorylated 200kDa NFP, SOX10, EMA, claudin-1, GLUT1, OCT4, KIT, CD30 loss, AFP loss, hCG, cytokeratin, inhibin, CD99, ETV6, WT1, SPDEF, CD56, β -catenin and others.

Practical and economical guidelines, which include a comprehensive list of IHC markers, for diagnosing CNS tumours in resource-restrained jurisdictions are being developed by the Asian Oceanian Society of Neuropathology for Adapting Diagnostic Approaches for Practical Taxonomy in Resource-Restrained Regions (AOSNP-ADAPTR).¹⁷²

To achieve ideal results, IHC should involve careful optimisation of antigen retrieval techniques and appropriate antibody selection. The inclusion of appropriate positive and negative controls, including on-slide controls, will ensure accurate and reliable results, enhance interpretation of staining patterns and minimize the risk of false-positive or false-negative findings.

* Only core for some tumours – refer to [Tables 1-3](#).

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Note 38 – Other molecular findings (Non-core)

These sections should be used for documenting findings for other genetic alterations and/or for other tumour types, such as metastases and haematological lesions.

 **Back**

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