Review Article



Molecular Testing of Central Nervous System Tumours: Recommendations of the Canadian Association of Neuropathologists

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ABSTRACT: The diagnosis of central nervous system tumours has been transformed in recent years from a microscopic morphology-based process to one dominated by the identification of somatic genetic alterations in tumour cells. This switch requires implementing radically different methods, for which appropriate training and financial resources must be allocated. The Canadian Association of Neuropathologists (CANP) has followed a process based on the scientific literature and consensus to develop recommendations for molecular testing of tumours of the brain and spinal cord, aiming to balance the need for treatment-determinant accurate diagnosis and the current limitations inherent in the transition to a new paradigm. The Professional Affairs Committee was charged with this task. A draft was discussed during the CANP general assembly, along with presentations from groups who had implemented molecular technologies, as well as others who relied on external laboratories. The Professional Affairs Committee summarised the consensus and submitted their recommendation to the CANP's Executive Committee. A final report was posted on the CANP website for a month to allow all members to comment. The recommendations below apply to intrinsic tumours of the central nervous system and do not include metastatic disease or tumours impinging upon the nervous system from outside. These recommendations should be considered clinically relevant, as the results have direct consequences on the patient's treatment, either through the use of targeted therapies or the trial-proven best application of radiation and/or chemotherapy.

RÉSUMÉ : Analyse moléculaire des tumeurs du système nerveux central : des recommandations de l'Association canadienne des neuropathologistes. Le diagnostic des tumeurs du système nerveux central est passé ces dernières années d'un processus basé sur la morphologie microscopique à un processus dominé par l'identification des altérations génétiques somatiques dans les cellules tumorales. Ce changement a nécessité la mise en oeuvre de méthodes radicalement différentes pour lesquelles une formation appropriée et des ressources financières doivent être allouées. C'est ainsi que l'Association canadienne des neuropathologistes (ACNP) s'est engagée dans un processus axé sur la littérature scientifique et l'établissement d'un consensus pour élaborer des recommandations portant sur les tests moléculaires des tumeurs du cerveau et de la moelle épinière, le tout visant à équilibrer le besoin d'un diagnostic précis et déterminant en vue d'un traitement et les limites actuelles inhérentes à la transition vers un nouveau paradigme. La commission des affaires professionnelles l'ACNP a été chargée de cette tâche. Un projet a donc été discuté lors de l'assemblée générale de l'ACNP. De plus, des groupes ayant mis en oeuvre des technologies moléculaires, ainsi que d'autres s'appuyant sur des laboratoires externes, ont effectué des présentations à cette occasion. Le comité des affaires professionnelles a résumé le consensus établi et a soumis ses recommandations au comité exécutif de l'ACNP. Un rapport final a ensuite été publié sur le site web de l'ACNP pendant un mois afin de permettre à tous les membres de faire part de leurs commentaires. Les recommandations ci-dessous s'appliquent aux tumeurs intrinsèques du système nerveux central et n'incluent pas les maladies métastatiques ou les tumeurs touchant le système nerveux de l'extérieur. Ces recommandations doivent aussi être considérées comme cliniquement pertinentes dans la mesure où les résultats ont des conséquences directes sur le traitement des patients, soit par une utilisation de thérapies ciblées, soit par une meilleure application de la radiothérapie et/ou de la chimiothérapie, lesquelles ont fait leurs preuves lors d'essais cliniques.

Keywords: Brain tumours; genetics – molecular biology; glioma; health services research; neuro-oncology; neuro-oncology (paediatric); neuro-oncology – medical

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Figure 1. Diffuse glioma diagnostic algorithm. 1p/19q = short arm of chromosome 1 and long arm of chromosome 19; ALK = anaplastic lymphoma kinase; ATRX = alpha-thalassaemia mental retardation X-linked; BRAF = v-raf murine sarcoma viral oncogene homologue B1; CDKN2A/B = cyclin-dependent kinase inhibitor 2A/B; CNV = copy number variants; EGFR = epidermal growth factor receptor; EZHIP = enhancer of zeste inhibitory protein; FGFR1 = fibroblast growth factor receptor 1; FISH = fluorescence in situ hybridisation; H3G34 = histone H3 mutation at codon 34; H3K27M = histone H3 mutation at codon 27; H3K27m3 = histone H3 trimethylation at lysine 27; IDH = isocitrate dehydrogenase; IHC = immunohistochemistry; LOH = loss of heterozygosity; MET = mesenchymal-epithelial transition; MGMT = 0 (6)-methylguanine-DNA-methyltransferase; MYB = myeloblastosis transcription factor; MYBL1 = MYB proto-oncogene like 1; MYCN = myelocytomatosis viral oncogene neuroblastoma-derived homologue; NGS = next-generation sequencing; NTRK1 = neurotrophic tyrosine kinase receptor; P53 = transformation-related protein 53; PDGFRA = platelet-derived growth factor receptor alpha; R132H = mutation in codon 132 of IDH1; ROS1 = c-ros oncogene 1; TERT = telomerase reverse transcriptase; wt = wildtype.

Introduction

The recommendations below provide an overview of best practices as of 2024 in the diagnosis of select tumours of the central nervous system including diffuse and circumscribed gliomas, glioneuronal tumours, ependymomas and medulloblastomas.^{1–8} These recommendations have been reviewed and approved at a national level by the Canadian Association of Neuropathologists (CANP). This document is expected to evolve over time in response to continued scientific study and clinical experience.

The document also serves to describe the minimum expectations for Canadian diagnostic laboratories to support the diagnosis of brain tumours to the current standards of the WHO, including the immunohistochemical, molecular and cytogenetic tests detailed below. For optimal workflow and timely patient care, it is recommended that decentralised testing be maximised where possible, although methylation profiling may require a more centralised approach.

The document was produced through the process described in the Abstract, combining the search for relevant scientific literature and consensus among the CANP members. The authors have been chosen to represent the diverse inputs, including the Professional Affairs Committee (DGM), the Executive Committee (AG), the president (RH) and secretary/treasurer of the association, a resident (MG) and a recognised leader in the field (CH).

Diffuse Gliomas

Diffuse gliomas are divided into adult-type and paediatric-type tumours. Adult-type diffuse gliomas include (1) isocitrate dehydrogenase (IDH)-mutant astrocytomas and oligodendrogliomas and (2) glioblastoma, IDH-wildtype. Paediatric-type diffuse gliomas are divided into (3) low-grade (characterised mainly by alterations in the genes in the MAPK pathway or MYB/MYBL1

genes) and (4) high-grade (characterised by a diverse array of alterations in histones, mismatch repair, receptor tyrosine kinases and others).^{2,9–12} Histologically, diffuse gliomas do not typically show distinctive features, so careful use of immunohistochemical and molecular testing is required to achieve an integrated diagnosis.

In adult patients, adult-type molecular alterations predominate. IDH-mutant gliomas should be distinguished from those that are IDH-wildtype, which have significantly poorer prognoses. Paediatric-type alterations should be considered in younger adults.^{2,5,9,12}

Please see Figure 1 for an adapted diagnostic flow chart for diffuse gliomas.

- The most common mutation in IDH1 (IDH1 p.R132H) may be assessed by immunohistochemistry (IHC). It can be combined with IHC for ATRX ± p53. In the context of an IDH mutation, ATRX loss (usually accompanied by aberrant p53 expression) is diagnostic of astrocytoma. Retained ATRX expression, on the other hand, should prompt testing for 1p/19q-codeletion, which is diagnostic of oligodendroglioma. The copy number status of CDKN2A/B may be assessed for grading of histologically lowergrade IDH-mutant astrocytomas. CDKN2A/B homozygous deletion has been linked to reduced survival and may serve as a molecular marker of grade 3 in oligodendrogliomas as well.^{1,2,12-14}
- 2. Tumours negative for IDH1 p.R132H may be assessed further depending on the clinical context.
 - i. In patients over the age of 55 years with a tumour showing glioblastoma histology, further diagnostic testing is generally unnecessary (glioblastoma, IDH-wildtype).^{9,11}
 - ii. In patients under the age of 55 years and/or with a tumour showing lower-grade histology, the tumour should be further assessed for "non-canonical" IDH1 and IDH2 mutations, and

as appropriate, molecular alterations associated with glioblastoma, IDH-wildtype (see recommendation 3) and/or paediatric-type alterations (see recommendations 5, 6 and 7) should also be considered.^{15,16}

- 3. Histologically, lower-grade IDH-wildtype diffuse gliomas, especially in those arising in middle-aged or older adults, should be tested for molecular alterations of glioblastoma, IDH-wildtype: combined chromosome Ch7 gain/Ch10 loss, epidermal growth factor receptor (EGFR) amplification and/or telomerase reverse transcriptase (TERT) promoter mutation.^{2,17-18}
- MGMT promoter methylation should be assessed in all glioblastoma, IDH-wildtype. It is at present unclear if IDHmutant astrocytoma patients would benefit from this test.^{5,8,19}

In children and younger adults, diffuse gliomas should be tested for paediatric-type alterations (see recommendations 6, 7 and 8). In paediatric and young adult patients, paediatric-type alterations are common and amenable to targeted therapy. Adult-type alterations (IDH1, IDH2) should be considered in patients of adolescent age and older. The location of the tumour (hemispheric vs. midline) also plays an important role in guiding testing.

- 5. Hemispheric diffuse low-grade gliomas should be assessed for alterations (SNVs, indels, fusions) in the MAPK pathway (including FGFR1, FGFR2, FGFR3, KRAS, NF1, BRAF), MYB and MYBL1. Less common alterations in NTRK1, NTRK2, NTRK3, MAP2K1 and MET may be included. Adult-type alterations should be considered. BRAF p.V600E can be assessed by IHC. BRAF p.V600E-mutant tumours should be assessed for CDKN2A/B copy number status. CDKN2A/B homozygous deletion and/or TERT and/or ATRX alterations should prompt consideration of an alternative diagnosis (see below: Circumscribed gliomas and glioneuronal tumours, recommendation 2).^{5–7,13,14}
- 6. Hemispheric diffuse high-grade gliomas should be assessed for mutations in H3-3A (diffuse hemispheric glioma, H3G34-mutant), SNVs and amplifications in EGFR and PDGFRA, alterations in genes involved in cancer predisposition syndromes (mismatch and replication repair [MLH1, MSH2, MSH6, PMS2, POLE, POLD1], TP53) and MYCN amplification. Adult-type alterations should be considered.^{5,7,20,21} There may be histologic overlap with tumours currently classified as "circumscribed" (see below: Circumscribed gliomas and glioneuronal tumours, recommendation 2). DNA methylation profiling may serve to distinguish between entities with overlapping molecular features. H3 p.G34R and MMR may be assessed by IHC. In infants, fusions involving receptor tyrosine kinases including ALK, ROS1, NTRK1, NTRK2, NTRK3 and MET should be assessed (infant-type hemispheric glioma).^{5,22}
- 7. Diffuse gliomas of the midline (thalamus, brainstem, cerebellum, spinal cord) in patients of all ages should be assessed for H3K27 trimethylation (H3K27me3) by IHC and for alterations in H3-3A (or less commonly, H3C2, H3C3 and H3C14). H3 p.K28M (K27M) can be assessed by IHC. Secondary alterations in BRAF or FGFR1 may be included. EGFR amplification and enhancer of zeste inhibitory protein (EZHIP) overexpression (IHC) should be assessed in H3-wildtype cases.^{20–22}

Circumscribed Gliomas and Glioneuronal Tumours

Most circumscribed gliomas and glioneuronal tumours are characterised by alterations (SNVs, indels, fusions) in the MAPK pathway. Copy number alterations in CDKN2A/B and alterations in genes in telomere maintenance (ATRX, TERT) are important additional alterations in particular tumour types.

Rare tumours in this category harbour specific molecular alterations. $^{\rm 14,22,23}$

- Pilocytic astrocytoma should be assessed for alterations in the MAPK pathway, including BRAF (SNVs and fusions), FGFR1 (SNVs, fusions, internal tandem duplication) and NF1. Testing may include less commonly altered genes such as KRAS, PTPN11 and RAF1.
- 2. High-grade astrocytoma with piloid features (HGAP) and pleomorphic xanthoastrocytoma (PXA) should be assessed for alterations in CDKN2A/B (copy number loss) and ATRX and/ or TERT, in addition to MAPK pathway alterations (BRAF, FGFR1, NF1). A matching DNA methylation profile is an essential diagnostic criterion for HGAP and desirable for PXA. HGAP can be highly favoured in the setting of RAS/MAPK alteration plus ATRX loss and/or CDKN2A/B homozygous deletion; however, definitive diagnosis currently requires methylation profiling.
- 3. Ganglioglioma should be assessed for alterations in BRAF. BRAF p.V600E may be assessed by IHC. Less commonly, alterations in other MAPK pathway genes including RAF1, KRAS and NF1 may be included. CDKN2A/B homozygous deletion should be absent.
- 4. The diagnosis of a variety of rare circumscribed gliomas and glioneuronal tumours may be confirmed by assessing for particular molecular alterations or by a specific DNA methylation profile. In these cases, next-generation sequencing is preferred over methylation profiling as it may provide the specific target for therapy.
 - Dysembryoplastic neuroepithelial tumour: FGFR1 SNVs, fusions, ITD
 - Papillary glioneuronal tumour: PRKCA fusions
 - Rosette forming glioneuronal tumour: FGFR1, NF1 and/or PIK3CA alterations
 - Myxoid glioneuronal tumour: PDGFRA SNVs
 - Diffuse leptomeningeal tumour: BRAF fusions with 1p loss ± 19q loss ± 1q gain
 - Multinodular vacuolating neuronal tumour: MAP2K1 SNVs
 - Extraventricular neurocytoma: FGFR1 fusions
 - Desmoplastic infantile ganglioglioma/astrocytoma: BRAF, RAF1, FGFR1 alterations
 - Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters: distinct DNA methylation profile
 - Astroblastoma: MN1 fusions
 - Chordoid glioma: PRKCA SNVs

Ependymoma

1. Posterior fossa ependymomas should be tested for H3K27me3 loss by IHC, along with EZHIP expression by IHC if possible, to distinguish posterior fossa group A (PFA) from posterior fossa group B (PFB) ependymoma. DNA methylation profiling is an alternative method that may be helpful in adult cases where PFB versus methylation class subependymoma is a more likely differential. PFA ependymoma should be assessed for copy number changes in 1q and 6q. Methylation class subependymoma should be assessed for TERT promoter mutation and chromosome 6 loss.^{24,25}
 Table 1.
 Summary of genes and molecular alterations in central nervous system neoplasms

	SNV/indel	Fusion	CNV
ALK		x	
ATRX	х		
BRAF	x	х	
CDKN2A/B			х
EGFR	х		х
FGFR1	х	х	
FGFR2	х	х	
FGFR3	х	х	
H3-3A	х		
H3C2	х		
IDH1	х		
IDH2	x		
KRAS	х		
MAP2K1	х		
MET	х	х	
MLH1	х		
MN1		х	
MSH2	х		
MSH6	х		
МҮВ		х	х
MYBL1		х	х
MYCN			х
NF1	х	х	
NTRK1	х	х	
NTRK2	х	х	
NTRK3	х	х	
PDGFRA	х		х
PIK3CA	х		
PMS2	х		
POLD1	х		
POLE	х		
PRKCA	х	х	
PTPN11	х		
RAF1		х	
ROS1		х	
TERT	х		
TP53	x		
YAP1		x	
ZFTA		x	
Ch 1			х
Ch 6			х
Ch 7			х

 Table 1. Summary of genes and molecular alterations in central nervous system

 neoplasms (Continued)

	SNV/indel	Fusion	CNV
Ch 10			х
Ch 19q			х

ALK = anaplastic lymphoma kinase; ATRX = alpha-thalassaemia mental retardation X-linked; BRAF = v-raf murine sarcoma viral oncogene homologue B1; CDKN2A/B = cyclin-dependent kinase inhibitor 2A/B; Ch = chromosome; CNV = copy number variants; EGFR = epidermal growth factor receptor; FGFR1/2/3 = fibroblast growth factor receptor 1/2/3; H3-3A = histone H3 variant H3.3; H3C2 = H3 clustered histone 2; IDH1/2 = isocitrate dehydrogenase 1/2; KRAS = Kirsten rat sarcoma viral oncogene homologue; MAPK21 = mitogen-activated protein kinase kinase 1; MET = mesenchymal-epithelial transition; MLH1 = human mutL homologue 1; MN1 = meningioma 1; MSH2/6 = MutS homologue 2/6; MYB = myeloblastosis transcription factor; MYBL1 = MYB proto-oncogene like 1; MYCN = myelocytomatosis viral oncogene neuroblastoma-derived homologue; NF1 = neurofibromatosis type 1; NTRK1/2/3 = neurotrophic tyrosine kinase receptor 1/2/3; PDGFRA = platelet-derived growth factor receptor alpha; PIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PMS2 = post-meiotic segregation increased 2; POLD1 = DNA polymerase delta 1; POLE = DNA polymerase epsilon; PRKCA = protein kinase C alpha; PTPN11 = tyrosine-protein phosphatase non-receptor type 11; q:long arm of chromosome; RAF1 = rapidly accelerated $fibrosarcoma; {\sf ROS1} = c\text{-}ros \ on cogene \ 1; \ {\sf SNV/indel} = single \ nucleotide \ variant/insertions \ and \$ deletions; TERT = telomerase reverse transcriptase; TP53 = tumour protein p53; YAP1 = yesassociated protein 1; ZFTA = zinc finger translocation associated.

Table 2. Essential immunohistochemical stains

IDH1 R132H
ATRX
P53
BRAF V600E
H3 K27M
H3 K27me3
H3 G34R
EZHIP
MLH1
MSH2
MSH6
PMS2

ATRX = alpha-thalassaemia mental retardation X-linked; BRAF V600E = v-raf murine sarcoma viral oncogene homologue B1 mutation in codon 600; H3G34 = histone H3 mutation at codon 34; H3 K27M = histone H3 mutation in codon 27; H3K27me3 = histone H3 trimethylation at lysine 27; IDH1 R132H = isocitrate dehydrogenase 1 mutation at codon 132; MLH1 = human mutL homologue 1; MSH2/6 = MutS homologue 2/6; PMS2 = post-meiotic segregation increased 2; PS3 = tumour protein p53.

Table 3. Tumour types in which the DNA methylation profile is included in WHO

 CNS 5e as an "essential" diagnostic criterion

Diffuse astrocytoma, MYB- or MYBL1-altered
Diffuse midline glioma, H3 K27-altered
Diffuse hemispheric glioma, H3 G34-mutant
Diffuse paediatric-type high-grade glioma, H3-wildtype and IDH- wildtype*
Infant-type hemispheric glioma

(Continued)

(Continued)

Table 3. Tumour types in which the DNA methylation profile is included in WHO

 CNS 5e as an "essential" diagnostic criterion (*Continued*)

Diffuse astrocytoma, MYB- or MYBL1-altered
High-grade astrocytoma with piloid features
Astroblastoma, MN1-altered
Ganglioglioma
Desmoplastic infantile ganglioglioma/desmoplastic infantile astrocytoma
Dysembryoplastic neuroepithelial tumour
Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters*
Papillary glioneuronal tumour
Diffuse leptomeningeal glioneuronal tumour
Extraventricular neurocytoma
Posterior fossa group A (PFA) ependymoma
Posterior fossa group B (PFB) ependymoma
Medulloblastoma, WNT-activated
Medulloblastoma, SHH-activated and TP53-wildtype
Medulloblastoma, SHH-activated and TP53-mutant
Medulloblastoma, non-WNT/non-SHH

*Asterisks identifies tumor types in which methylation profiling is the only method to reach a diagnosis.

Table 4. Summary of gliomas and glioneuronal tumours

- Supratentorial ependymomas should be tested for ZFTA and YAP1 fusions. In those with ZFTA fusion, CDKN2A/B copy number status should be assessed.
- 3. Spinal cord ependymomas with high-grade histology should be assessed for MYCN amplification.

Medulloblastoma

- 1. All medulloblastomas should undergo molecular testing to determine the molecular subgroup. DNA methylation profiling and/or NanoString analysis are suitable techniques. Assessment of copy number alterations may also be indicated depending on subgroup (e.g. MYC amplification in group 3).²⁵
- 2. SHH-activated medulloblastoma in the paediatric age group should be assessed for TP53 alterations.

The tables summarise the molecular alterations (Table 1), crucial immunohistochemical stains (Table 2) and tumour types for which the DNA methylation profile is considered an "essential" diagnostic criterion by WHO (Table 3). In Table 3, we highlight with an asterisk those tumour types for which methylation profiling is the only method to reach a diagnosis. For all other tumour types in this table, next-generation sequencing represents an alternative approach. In addition, any unresolved cases could

	Glioblastoma, IDH-wildtype	IDH-mutant gliomas	Paediatric-type diffuse low-grade gliomas	Paediatric-type diffuse high-grade gliomas	Circumscribed gliomas and GNT	Ependymoma	Medulloblastoma
IHC		IDH1R132H, ATRX, p53	BRAFV600E	H3K27M, H3K27me3, EZHIP, H3G34R, MMR (MLH1, MSH2, MSH6, PMS2), p53	BRAFV600E	H3K27me3	P53
SNV/indel	TERT	IDH1, IDH2, ATRX, TP53	BRAF, FGFR1, KRAS, MAP2K1, MET, NF1	EGFR, H3-3A, H3C2, MLH1, MSH2, MSH6, PDGFRA, PMS2, POLD1, POLE, TP53	ATRX, BRAF, KRAS, FGFR1, MAP2K1, NF1, PDGFRA, PIK3CA, PRKCA, PTPN11, TERT	TERT	TP53
Fusion			BRAF, FGFR1, FGFR2, FGFR3, NTRK1, NTRK2, NTRK3, MYB, MYBL1	ALK, MET, NTRK1, NTRK2, NTRK3, ROS1	BRAF, FGFR1, MN1, PRKCA, RAF1	ZFTA, YAP1	
CNV	EGFR	CDKN2A/B	MYB, MYBL1	EGFR, PDGFRA, MYCN	CDKN2A/B	CDKN2A/B, MYCN	
Cytogenetics	7, 10	1p, 19q			1p, 1q, 19q	1q, 6	
DNA methylation profiling			Essential (some tumour types)	Essential (some tumour types)	Essential (some tumour types)	Essential (some tumour types)	Essential (or NanoString)
Other	MGMT promoter methylation						

ALK = anaplastic lymphoma kinase; ATRX = alpha-thalassaemia mental retardation X-linked; BRAF = v-raf murine sarcoma viral oncogene homologue B1; CDKN2A/B = cyclin-dependent kinase inhibitor 2A/B; CNV = copy number variants; EGFR = epidermal growth factor receptor; EZHIP = enhancer of zeste inhibitory protein; FGFR1/2/3 = fibroblast growth factor receptor 1/2/3; H3-3A = histone H3 variant H3.3; H3C2 = H3 clustered histone 2; H3G34 = histone H3 mutation at codon 34; H3K27M = histone H3 mutation at codon 27; H3K27ma3 = histone H3 trimethylation at lysine 27; IDH 1/2 = isocitrate dehydrogenase 1/2; IHC = immunohistochemistry; MAP2K1 = mitogen-activated protein kinase kinase 1; MET = mesenchymal-epithelial transition; MLH1 = human mutL homologue 1; MN1 = meningioma 1; MSH2/6 = MutS homologue 2/6; MGMT = O (6)-methylguanine-DNA-methyltransferase; MMR = mismatch repair MYB = myeloblastosis transcription factor; MYBL1 = MYB proto-oncogene like 1; MYCN = myelocytomatosis viral oncogene neuroblastoma-derived homologue; NF1 = neurofibromatosis type 1; NTRK1/2/3 = neurotrophic tyrosine kinase receptor; PS3 = transformation-related protein 53; *p* = short arm of a chromosome; *q* = long arm of a chromosome, PDGFRA = platelet-derived growth factor receptor alpha; PIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PMS2 = post-meiotic segregation increased 2; POLD1 = DNA polymerase delta 1; POLE = DNA polymerase epsilon; PRKCA = protein kinase C α ; PTPN11 = tyrosine-protein phosphatase non-receptor type 11; RAF1 = rapidly accelerated fibrosarcoma; ROS1 = c-ros oncogene 1; TERT = telomerase reverse transcriptase; TP53 = tumour protein potein p53; YAP1 = yes-associated protein-1; ZFTA = zinc finger translocation associated.

benefit from methylation analysis. Table 4 summarises the defining molecular alterations in gliomas and glioneuronal tumours.

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References

- Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Dev Oncol.* 2021; 23:1231–51. DOI: 10.1093/neuonc/noab106.
- Brat DJ, Aldape K, Bridge JA, et al. Molecular biomarker testing for the diagnosis of diffuse gliomas. *Arch Pathol Lab Med.* 2022;146:547–74. DOI: 10.5858/arpa.2021-0295-CP.
- Horbinski C, Ligon KL, Brastianos P, et al. The medical necessity of advanced molecular testing in the diagnosis and treatment of brain tumor patients. *Neuro Oncol.* 2019;21:1498–508. DOI: 10.1093/neuonc/noz119.
- 4. Komori T. Beyond the WHO 2021 classification of the tumors of the central nervous system: transitioning from the 5th edition to the next. *Brain Tumor Pathol.* 2021;41:1–3. DOI: 10.1007/s10014-023-00474-5.
- Lim-Fat MJ, Macdonald M, Lapointe S, et al. Molecular testing for adolescent and young adult central nervous system tumors: a Canadian guideline. *Front Oncol.* 2022;12:960509. DOI: 10.3389/fonc.2022.960509.
- Han PC, Baker TG. Glial and glioneuronal tumors: navigating the complexity of evolving concepts and new classification. *J Neurol Sci.* 2024; 461:123058. DOI: 10.1016/j.jns.2024.123058.
- Stone TJ, Mankad K, Tan AP, et al. DNA methylation-based classification of glioneuronal tumours synergises with histology and radiology to refine accurate molecular stratification. *Neuropathol Appl Neurobiol.* 2023;49: e12894. DOI: 10.1111/nan.12894.
- Farrell C, Shi W, Bodman A, Olson JJ. Congress of neurological surgeons systematic review and evidence-based guidelines update on the role of emerging developments in the management of newly diagnosed glioblastoma. J Neurooncol. 2020;150:269–359. DOI: 10.1007/s11060-020-03607-4.
- Zarnett OJ, Sahgal A, Gosio J, et al. Treatment of elderly patients with glioblastoma: a systematic evidence-based analysis. *JAMA Neurol.* 2015;72:589–96. DOI: 10.1001/jamaneurol.2014.3739.
- 10. Goodman AL, Velázquez Vega JE, Glenn C, Olson JJ. Congress of neurological surgeons systematic review and evidence-based guidelines

update on the role of neuropathology in the management of progressive glioblastoma in adults. *J Neurooncol.* 2022;158:179–224. DOI: 10.1007/ s11060-022-04005-8.

- Yuen CA, Barbaro M, Haggiagi A. Newly diagnosed glioblastoma in elderly patients. *Curr Oncol Rep.* 2022;24:325–34. DOI: 10.1007/s11912-022-01201-7.
- Rousseau J, Bennett J, Lim-Fat MJ. Brain tumors in adolescents and young adults: a review. *Semin Neurol.* 2023;43:909–28. DOI: 10.1055/s-0043-1776775.
- Jonsson P, Lin AL, Young RJ, et al. Genomic correlates of disease progression and treatment response in prospectively characterized gliomas. *Clin Cancer Res.* 2019;25:5537–47. DOI: 10.1158/1078-0432. CCR-19-0032.
- Galbraith K, Garcia M, Wei S, et al. Prognostic value of DNA methylation subclassification, aneuploidy, and CDKN2A/B homozygous deletion in predicting clinical outcome of IDH mutant astrocytomas. *Neuro Oncol.* 2024;26:1042–51. DOI: 10.1093/neuonc/noae009.
- Berger TR, Wen PY, Lang-Orsini M, Chukwueke UN. World Health Organization 2021 classification of central nervous system tumors and implications for therapy for adult-type gliomas: a review. *JAMA Oncol.* 2022;8:1493–501. DOI: 10.1001/jamaoncol.2022.2844.
- Mohile NA, Messersmith H, Gatson NT, et al. Therapy for diffuse astrocytic and oligodendroglial tumors in adults: ASCO-SNO guideline. *J Clin Oncol.* 2036;40:403–426. DOI: 10.1200/JCO.21.21.02036.
- Berzero G, Di Stefano AL, Ronchi S, et al. IDH-wildtype lower-grade diffuse gliomas: the importance of histological grade and molecular assessment for prognostic stratification. *Neuro Oncol.* 2021;23:955–66. DOI: 10.1093/ neuonc/noaa258.
- Capper D, Reifenberger G, French PJ, et al. EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection. *Neuro Oncol.* 2023;25:813–26. DOI: 10.1093/ neuonc/noad008.
- Melhem JM, Detsky J, Lim-Fat MJ, Perry JR. Updates in IDH-wildtype glioblastoma. *Neurotherapeutics*. 2022;19:1705–23. DOI: 10.1007/s13311-022-01251-6.
- 20. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol.* 2012;124:439–47. DOI: 10.1007/s00401-012-0998-0.
- Schwartzentruber J, Korshunov A, Liu XY, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012;482:226–31. DOI: 10.1038/nature10833.
- Rudà R, Capper D, Waldman AD, et al. EURACAN SNO guidelines on circumscribed astrocytic gliomas, glioneuronal, and neuronal tumors. *Neuro Oncol.* 2022;24:2015–34. DOI: 10.1093/neuonc/noac188.
- Sahm F, Brandner S, Bertero L, et al. Molecular diagnostic tools for the World Health Organization (WHO) 2021 classification of gliomas, glioneuronal and neuronal tumors; an EANO guideline. *Neuro Oncol.* 2023;25:1731–49. DOI: 10.1093/neuonc/noad100.
- 24. Gillen AE, Riemondy KA, Amani V, et al. Updates in the classification of ependymal neoplasms: the 2021 WHO classification and beyond. *Brain Pathol.* 2022;32:e13068. DOI: 10.1111/bpa.13068.
- Northcott PA, Buchhalter I, Morrissy AS, et al. The whole-genome landscape of medulloblastoma subtypes. *Nature*. 2017;547:311–7. DOI: 10.1038/nature22973.