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

David G Munoz, Andrew Gao, Robert Hammond, Peter W. Schutz, Madison Gray, Cynthia Hawkins

1. Department of Laboratory Medicine, Unity Health Toronto, Toronto, ON; David.Munoz@unityhealth.to; 4168645858
2. Laboratory Medicine Program, University Health Network, Toronto, ON; Andrew.Gao@uhn.ca; 416-340-3105
3. Department Pathology and Laboratory Medicine, London Health Sciences Center, London, ON; Robert.Hammond@lhsc.on.ca; 519 685 8500 x36367
4. Division of Neuropathology, Vancouver General Hospital, Vancouver, BC; Peter.Schutz@vch.ca; 604-875-4111 x 21106
5. Clinical Department of Laboratory Medicine, Centre Hospitalier de l'Université de Montréal (CHUM), Montreal, Quebec madison.gray.med@ssss.gouv.qc.ca 514-890-8000
6 Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON; cynthia.hawkins@sickkids.ca; 416-813-5938

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Statement of authorship

All authors have participated on discussions regarding the content and in the preparation of the manuscript. In addition, DGM chaired the Professional Affairs Committee and wrote the first draft, AG extensively edited the manuscript and wrote the algorithm and tables, RH originated the idea of preparing the recommendations and presided over the process, PWS organized the meeting where the recommendations were discussed, MG contributed to the discussion and editing of the manuscript, CH provided general guidance and specific editing.

Abstract

The diagnosis of central nervous system tumors has been transformed in recent years from a microscopic morphology-based process to one dominated by the identification of somatic genetic alterations in tumor 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 developed recommendations for molecular testing of tumors of the brain and spinal cord, aiming to balance the need of treatment-determinant accurate diagnosis and the current limitations inherent to 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 summarized 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 tumors of the central nervous system, and do not include metastatic disease or tumors impinging upon the nervous system from outside. These recommendations should be considered clinically relevant, as the results have direct consequences on patient's treatment, either through the use of targeted therapies or the trial-proven best application of radiation and/or chemotherapy.

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The recommendations below provide an overview of best practices as of 2024 in the diagnosis of select tumours of the CNS 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 de-centralized testing be maximized where possible, although methylation profiling may require a more centralized approach.

The document was produced through the process described in the Abstract, combining the search of relevant scientific literature and consensus among the CANP membership. The authors have been chosen to represent the diverse inputs, representing the Professional Affairs Committee (DGM), the Executive Committee (AG), the President (RH) and Secretary/Treasurer (PS) of the association, a resident (MG), and a recognized leader in the field (CH).

Diffuse gliomas

Diffuse gliomas are divided into adult-type and pediatric-type tumours. Adult-type diffuse gliomas include (1) IDH-mutant astrocytomas and oligodendrogliomas and (2) glioblastoma, IDH-wildtype. Pediatric-type diffuse gliomas are divided into (3) low-grade (characterized mainly by alterations in the genes in the MAPK pathway or MYB/MYBL1 genes) and (4) high-grade (characterized 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, and so discerning 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. Pediatric type alterations should be considered in younger adults [2,5,9,12].

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

1. 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 should prompt testing for 1p/19q-codeletion, diagnostic of oligodendroglioma. Copy number status of CDKN2A/B may be assessed for grading of histologically lower-grade 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 55y with a tumour showing glioblastoma histology, further diagnostic testing is generally unnecessary (glioblastoma, IDH-wildtype) [9,11].

    ii. In patients under 55y 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 of glioblastoma, IDH-wildtype (see recommendation 3) and/or pediatric-type alterations (see recommendations 5, 6 and 7) [15,16].

3. Histologically lower-grade IDH-wildtype diffuse gliomas, especially in those arising in middle-age or older adults, should be tested for molecular alterations of glioblastoma, IDH-wildtype: combined chromosome Ch7 gain/Ch10 loss, EGFR amplification, and/or TERT promoter mutation [2,17-18].

4. MGMT promoter methylation should be assessed in all glioblastoma, IDH-wildtype. It is at present unclear if IDH-mutant astrocytoma patients would benefit from this test [5,8,19].

In Children and younger adults' diffuse gliomas should be tested for pediatric-type alterations (see recommendations 6, 7, and 8). In pediatric and young adult patients, pediatric-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.

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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 immunohistochemistry. 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 immunohistochemistry. Secondary alterations in BRAF or FGFR1 may be included. EGFR amplification and EZHIP overexpression (IHC) should be assessed in H3-wildtype cases [20-22].

Circumscribed gliomas and glioneuronal tumours

Most circumscribed gliomas and glioneuronal tumours are characterized 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 [14,22,23].

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1. 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 although currently, definitive diagnosis 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

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Ependymoma

1. Posterior fossa ependymomas should be tested for H3K27me3 loss by IHC, along with EZHIP expression by IHC if possible, to distinguish PFA from PFB ependymoma. DNA methylation profiling is an alternative method that may be helpful in adult cases where PFB vs 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].

2. 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) [26].

2. SHH-activated medulloblastoma in the pediatric age group should be assessed for TP53 alterations.

The Tables summarize the molecular alterations (Table 1), crucial immunohistochemical stains (Table 2), tumour types in which the DNA methylation profile is considered an “essential” diagnostic criterion by WHO (Table 3), in which we identify by an asterisk those in which methylation profiling is the only method to reach a diagnosis. In all other tumor types in this table Next Generation Sequencing represents an alternative approach. In addition, any unresolved cases could benefit from methylation analysis. Table 4 summarizes the defining molecular alterations in gliomas and glioneuronal tumours.

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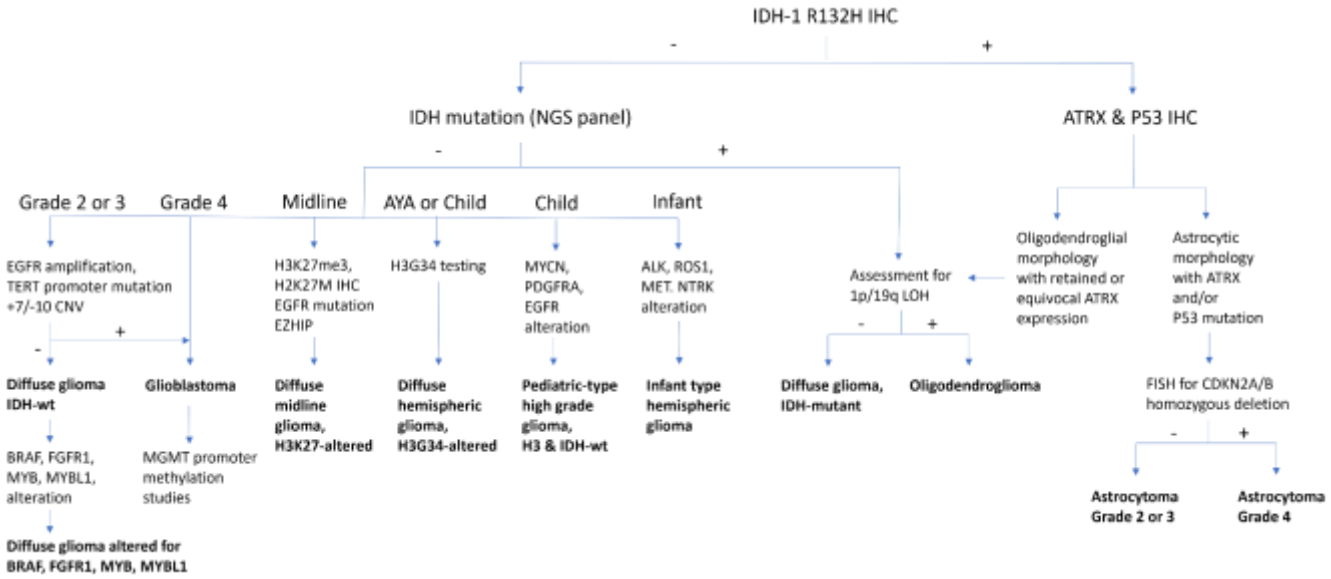
Figure 1: Diffuse Glioma Diagnostic Algorithm



Abbreviations.

1p/19q: short arm of chromosome 1 and long arm of chromosome 19, ALK: anaplastic lymphoma kinase, ATRX: alpha-thalassemia mental retardation X-linked, BRAF: v-raf murine sarcoma viral oncogene homolog 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 hybridization, H3G34: histone H3 mutation at codon 34, H3K27M: histone H3 mutation at codon 27, H3K27me3: histone H3 trimethylation at lysine 27, IDH: isocitrate dehydrogenase, IHC: Immunohistochemistry, LOH: loss of heterozygosity, MET: Mesenchymal Epithelial Transition, MGMT: O(6)-Methylguanine-DNA-methyltransferase, MYB: myeloblastosis transcription factor, MYBL1: MYB proto-oncogene like 1, MYCN: myelocytomatosis viral oncogene neuroblastoma derived homolog, 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.

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Table 1: Summary of genes and molecular alterations in CNS neoplasms

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

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TP53	x		
YAP1		x	
ZFTA		x	
Ch 1			x
Ch 6			x
Ch 7			x
Ch 10			x
Ch 19q			x

Abbreviations.

ALK: anaplastic lymphoma kinase, ATRX: alpha-thalassemia mental retardation X-linked, BRAF: v-raf murine sarcoma viral oncogene homolog 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 homolog, MAPK21: mitogen-activated protein kinase kinase 1, MET: Mesenchymal Epithelial Transition, MLH1: Human mutL homolog 1, MN1: Meningioma 1, MSH2/6: MutS homolog 2/6, MYB: myeloblastosis transcription factor, MYBL1: MYB proto-oncogene like 1, MYCN: myelocytomatosis viral oncogene neuroblastoma derived homolog, 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, ROS1: c-ros oncogene 1, SNV/indel: single nucleotide variant/insertions and deletions, TERT: telomerase reverse transcriptase, TP53: tumor protein p53, YAP1: yes-associated protein 1, ZFTA: zinc finger translocation associated

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Table 2: Essential immunohistochemical stains

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

Abbreviations.

ATRX: alpha-thalassemia mental retardation X-linked, BRAF V600E: v-raf murine sarcoma viral oncogene homolog 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 homolog 1, MSH2/6: MutS homolog 2/6, PMS2: post-meiotic segregation increased 2, P53: tumor protein p53

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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
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

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Table 4. Summary of gliomas and glioneuronal tumours

	Glioblastoma, IDH-wildtype	IDH-mutant gliomas	Pediatric-type diffuse low grade gliomas	Pediatric-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	

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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						

Abbreviations.

ALK: anaplastic lymphoma kinase, ATRX: alpha-thalassemia mental retardation X-linked, BRAF: v-raf murine sarcoma viral oncogene homolog 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, H3K27me3: 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 homolog 1, MN1: Meningioma 1, MSH2/6: MutS homolog 2/6, MET: Mesenchymal Epithelial Transition, 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 homolog, NF1: Neurofibromatosis type 1, NTRK1/2/3: neurotrophic tyrosine kinase receptor, P53: 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: tumor protein p53, YAP1: yes-associated protein-1, ZFTA: zinc finger translocation associated

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