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Molecular Testing for the World Health Organization Classification of Central Nervous System Tumors
A Review

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IMPORTANCE Molecular techniques, including next-generation sequencing, genomic copy number profiling, fusion transcript detection, and genomic DNA methylation arrays, are now indispensable tools for the workup of central nervous system (CNS) tumors. Yet there remains a great deal of heterogeneity in using such biomarker testing across institutions and hospital systems. This is in large part because there is a persistent reluctance among third-party payers to cover molecular testing. The objective of this Review is to describe why comprehensive molecular biomarker testing is now required for the accurate diagnosis and grading and prognostication of CNS tumors and, in so doing, to justify more widespread use by clinicians and coverage by third-party payers.

OBSERVATIONS The 5th edition of the World Health Organization (WHO) classification system for CNS tumors incorporates specific molecular signatures into the essential diagnostic criteria for most tumor entities. Many CNS tumor types cannot be reliably diagnosed according to current WHO guidelines without molecular testing. The National Comprehensive Cancer Network also incorporates molecular testing into their guidelines for CNS tumors. Both sets of guidelines are maximally effective if they are implemented routinely for all patients with CNS tumors. Moreover, the cost of these tests is less than 5% of the overall average cost of caring for patients with CNS tumors and consistently improves management. This includes more accurate diagnosis and prognostication, clinical trial eligibility, and prediction of response to specific treatments. Each major group of CNS tumors in the WHO classification is evaluated and how molecular diagnostics enhances patient care is described.

CONCLUSIONS AND RELEVANCE Routine advanced multidimensional molecular profiling is now required to provide optimal standard of care for patients with CNS tumors.

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Central nervous system (CNS) tumors are the most common kind of solid tumor diagnosed in children, the second most common in adolescents, and the eighth most common in adults.[1,2] CNS tumors rank first among all cancers in terms of average years of life lost.[3] One of the most common CNS tumors, gliomas, is among the most expensive cancers to treat.[4,5] Accurate diagnoses are essential to direct costly treatments for patients who need them the most.

The newest edition of the World Health Organization (WHO) classification CNS tumors includes tumors that now require detection of specific molecular alterations (**Table**). When integrated with histology, the entire spectrum of CNS tumors can now be diagnosed using 1 or more molecular tests, including next-generation sequencing (NGS), genomic copy number array, fusion screening, and genomic DNA methylation profiling. However, third-party payers are often still reluctant to cover such testing. In a 2024 study,[6] coverage of NGS was denied in 77% of cancer cases, with only 10.8% reimbursement, even though NGS improved clinical management most of the time. This represents a persistent barrier for patients and clinicians.

This Review explains the rationale for multidimensional molecular diagnostics for CNS tumors, as articulated in the WHO classification and in the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. This provides third-party payers with evidence-based justification for covering molecular testing. The underlying principles for each molecular platform and the NCCN Guidelines can be found in eFigures 1 to 14 in the Supplement. A description of common molecular testing approaches can be found in the eMethods in the Supplement.

Table. Diagnostic and Prognostic Molecular Markers Among Major Primary Central Nervous System (CNS) Tumors[a]

CNS tumor	Diagnostic or prognostic molecular features
Gliomas, glioneuronal tumors, and neuronal tumors	
Adult-type diffuse gliomas	
Astrocytoma, IDH-mutant	Alterations in *IDH1* or *IDH2*, *ATRX*, and *TP53*; *CDKN2A/B* deletion, *CDK4* and *PDGFRA* amplification
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	Alterations in *IDH1* or *IDH2*, *TERT* promoter, *CIC*, *FUBP1*, *NOTCH1*; whole-arm 1p/19q codeletion
Glioblastoma, IDH wild-type	IDH wild-type, *TERT* promoter, chromosomes 7 gain and 10 loss, *EGFR* amplification, *CDKN2A/B* deletion, *PTEN* inactivation; *MGMT* promoter methylation
Pediatric-type diffuse low-grade gliomas	
Diffuse astrocytoma, MYB-altered or MYBL1-altered[b]	Methylation profiling, fusions involving *MYB* or *MYBL1*
Angiocentric glioma	Methylation profiling, *MYB::QKI* fusion
Polymorphous low-grade neuroepithelial tumor of the young[b]	Methylation profiling, MAPK pathway-activating alterations, eg, *BRAF* V600E, *FGFR2* fusion
Diffuse low-grade glioma, MAPK pathway-altered[b]	Methylation profiling, MAPK pathway-activating alterations, usually *FGFR1* or *BRAF*
Pediatric-type diffuse high-grade gliomas	
Diffuse midline glioma, H3 K27-altered	Methylation profiling, alteration in H3.1, 3.2, or 3.3 K27; *EGFR* variant; EZH inhibitory protein overexpression
Diffuse hemispheric glioma, H3 G34-mutant[b]	Alteration at H3.3 G34, usually with *TP53* and *ATRX* variants; *PDGFRA* variant
Diffuse pediatric-type high-grade glioma, H3 wild-type, and IDH wild-type[b]	Methylation profiling, activating alterations of *PDGFRA*, *MYCN*, or *EGFR*; also can have alterations in *TP53*, *NF1*, and/or *TERT* promoter
Infant-type hemispheric glioma[b]	Most have isolated fusions in RTK-encoding genes, eg, *ALK*, *ROS1*, *MET*, *NTRK1-3*
Circumscribed astrocytic gliomas	
Pilocytic astrocytoma	Activating variant or fusion in *BRAF* or other MAPK pathway genes
High-grade astrocytoma with piloid features[b]	Methylation profiling; MAPK-activating alterations plus *ATRX*-inactivating and *CDKN2A/B*-inactivating alterations
Pleomorphic xanthoastrocytoma	Methylation profiling, MAPK-activating alterations, mainly *BRAF* V600E; *CDKN2A/B* inactivation
Subependymal giant cell astrocytoma	Alteration in *TSC1* or *TSC2*
Chordoid glioma	*PRKCA* D463H alteration
Astroblastoma, *MN1*-altered	Methylation profiling, *MN1::BEND2* or *EWSR1::BEND2* fusions
Glioneuronal and neuronal tumors	
Ganglion cell tumors (gangliоglioma, gangliocytoma)	Mainly *BRAF* V600E or other *BRAF* variants/rearrangements, methylation profiling
Desmoplastic infantile gangliоglioma/astrocytoma	Methylation profiling, MAPK-activating alterations, mainly involving *BRAF* or *RAF1*
Dysembryoplastic neuroepithelial tumor	Methylation profiling, *FGFR1* variant or kinase domain tandem duplication
Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters[b]	Methylation profiling, chromosome 14 monosomy
Papillary glioneuronal tumor	Methylation profiling, *PRKCA* fusions
Rosette-forming glioneuronal tumor	Methylation profiling, *FGFR1* N546 or K656 plus *PIK3CA/PIK3R1* variants
Myxoid glioneuronal tumor[b]	Methylation profiling, *PDGFRA* variant, mainly K385L or K385I
Diffuse leptomeningeal glioneuronal tumor	Methylation profiling, 1p deletion plus *KIAA1549::BRAF* fusion
Multinodular and vacuolating neuronal tumor[b]	MAPK pathway-activating alterations, mainly *MAP2K1* and *BRAF* variants
Dysplastic cerebellar gangliocytoma	*PTEN* inactivation
Neurocytomas (central, extraventricular, cerebellar)	Methylation profiling for all 3 subtypes, *FGFR1::TACC1* fusions in extraventricular subtype
Ependymal tumors	
Supratentorial ependymoma, *ZFTA* fusion-positive	*ZFTA* fusions; *CDKN2A/B* deletion
Supratentorial ependymoma, *YAP1* fusion-positive[b]	*YAP1* fusions
Posterior fossa ependymoma group PFA[b]	Methylation profiling
Posterior fossa ependymoma group PFB[b]	Methylation profiling
Spinal ependymoma	*NF2* inactivation
Spinal ependymoma, *MYCN*-amplified[b]	*MYCN* (or *MYC*) amplification
Myxopapillary ependymoma	Methylation profiling, +chromosome 16/−chromosome 10
Subependymoma	Methylation profiling, infratentorial tumors often have −chromosome 19 and −chromosome 6

(continued)

Table. Diagnostic and Prognostic Molecular Markers Among Major Primary Central Nervous System (CNS) Tumors[a] (continued)

CNS tumor	Diagnostic or prognostic molecular features
Embryonal tumors	
Medulloblastoma	
Medulloblastoma, WNT-activated	Methylation profiling, *CTNNB1* alterations
Medulloblastoma, SHH-activated, *TP53* wild-type	Methylation profiling, SHH pathway variants, *TP53* wild-type
Medulloblastoma, SHH-activated, *TP53*-mutant	Methylation profiling, SHH pathway variants, *TP53* mutant
Medulloblastoma, non-WNT/non-SHH	Methylation profiling, alterations in *MYC*, *MYCN*, *PRDM6*, *KBTBD4*, *KDM6A*; various CNAs
Other CNS embryonal tumors	
Atypical teratoid/rhabdoid tumor	Methylation profiling, *SMARCB1*, *SMARCA4* alterations
Embryonal tumor with multilayered rosettes	Methylation profiling, C19MC structural alterations, *DICER1* alterations
CNS neuroblastoma, *FOXR2*-activated[b]	Methylation profiling, *FOXR2* rearrangements
CNS tumor with *BCOR* internal tandem duplication[b]	Methylation profiling, *BCOR* internal tandem duplication
Pineal tumors	
Pineocytoma	Methylation profiling
Pineal parenchymal tumor of intermediate differentiation	Methylation profiling, *KBTBD4* in-frame insertions
Pineoblastoma	Methylation profiling; alterations in *DICER1*, *DROSHA*, or *DGCR8*; *RB1* alterations; *MYC* amplification
Papillary tumor of the pineal region	Methylation profiling
Desmoplastic myxoid tumor of the pineal region, *SMARCB1*-mutant	Methylation profiling, *SMARCB1* alterations
Meningiomas	Alterations in *NF2*, *AKT1*, *TRAF7*, *SMO*, *PIK3CA*, *KLF4*, *SMARCE1*, or *BAP1* in various subtypes; *TERT* promoter variant or homozygous *CDKN2A/B* deletion in CNS WHO grade 3; other recurrent CNAs (eg, 1p and 10q) associated with shorter PFS

Abbreviations: CNA, copy number alteration; IDH, isocitrate dehydrogenase; MAPK, mitogen-activated protein kinase; PFA, posterior fossa type A; PFB, posterior fossa type B; PFS, progression-free survival; RTK, receptor tyrosine kinase; WHO, World Health Organization.

[a] This is not a comprehensive list of all tumor types in the 5th edition of the World Health Organization CNS classification. Each listed tumor type and subtype can be differentiated from each other by methylation profiling.

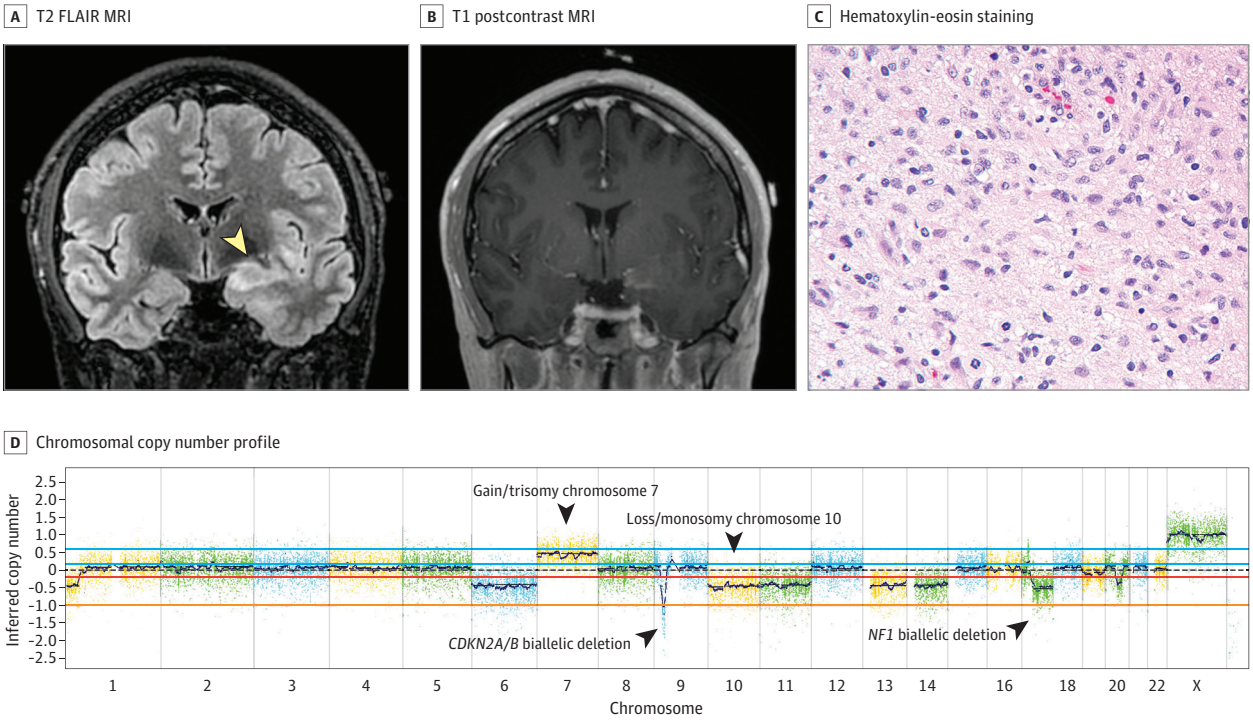
[b] Newly recognized tumors.

Adult-Type Diffuse Gliomas

The 5th edition of the WHO classification recognizes 3 subsets of adult-type diffuse gliomas: isocitrate dehydrogenase (IDH)–mutant astrocytoma, CNS WHO grades 2 to 4; IDH-mutant and 1p/19q-codeleted oligodendroglioma, CNS WHO grades 2 to 3; and IDH wild-type glioblastoma, CNS WHO grade 4. Since IDH-mutant astrocytomas with homozygous *CDKN2A/B* deletion behave like grade 4 IDH-mutant astrocytomas whether or not necrosis and/or microvascular proliferation are visible microscopically, homozygous *CDKN2A/B* deletion is now sufficient to render an IDH-mutant astrocytoma grade 4. The term *glioblastoma* should only be used when a diffusely infiltrative IDH wild-type and histone H3 wild-type astrocytic glioma has necrosis and/or microvascular proliferation and/or 1 or more of the following molecular alterations: *TERT* promoter mutation, *EGFR* amplification, or concomitant gain of chromosome 7 and loss of chromosome 10 (or just 10q).[7] Gliomas with only lower-grade histologic features can be upgraded to CNS WHO grade 4 glioblastomas by these molecular criteria; thus, routine screening of all gliomas for these markers is warranted.[8] Tumors that microscopically look like glioblastoma and have negative immunohistochemistry findings for the canonical IDH1 R132H variant may contain a noncanonical IDH variant (eg, R132C, R132G) that can only be detected by sequencing.[9] At Northwestern Memorial Hospital, for example, 11 of 93 IDH-mutant grade 4 astrocytomas (12%) have such a noncanonical variant. Tumors resembling glioblastoma may have genetic alterations and DNA methylation profiles that change the diagnosis to circumscribed astrocytomas, pediatric-type high-grade diffuse gliomas, or CNS embryonal tumors.[10] Conversely, gliomas that histologically look like oligodendroglioma, pleomorphic xanthoastrocytoma (PXA), or some other lower-grade neoplasm may ultimately be diagnosed as glioblastoma based on their mutation, copy number, and DNA methylation profiles (**Figure 1** and **Figure 2**).[10,11]

Accurate, reliable classification of adult-type diffuse gliomas has enormous clinical implications. This new molecular system allows for more consistent prognoses across institutions, reduces variation in treatment responses and overall outcomes, and makes clinical trial results more robust. As indicated in the NCCN Guidelines, "NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged."[12] Standard treatment for gliomas meeting the molecular criteria for glioblastoma are fractionated radiotherapy (RT) and concurrent temozolomide followed by adjuvant temozolomide[13]; electromagnetic tumor-treating fields may also be considered.[14] Based on clinical trial data, glioblastoma treatment recommendations vary if the patient is older than 70 years, has a Karnofsky performance status score less than 70, and/or *MGMT* promoter is unmethylated.[15,16] As concerns IDH-mutant astrocytomas and oligodendrogliomas, a major shift is currently underway based on the recent phase 3 INDIGO trial in which patients with nonenhancing CNS WHO grade 2 IDH-mutant astrocytomas and oligodendrogliomas showed substantial improvement in progression-free survival and time to next intervention when treated with the pan–IDH-mutant inhibitor vorasidenib.[17] A separate retro-

Figure 1. Requirement of Advanced Molecular Testing to Accurately Classify and Treat Diagnostically Ambiguous Gliomas, Part 1



A woman in her late 40s presented with new onset of aphasia and left arm paresthesia. Preoperative T2-weighted fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) showed a hyperintense lesion with minimal enhancement on postcontrast imaging centered in the left mesial temporal lobe and insula (arrowhead). Surgical resection of the lesion was performed that revealed a histologically low-grade glial or glioneuronal neoplasm (original magnification ×100), for a which a consensus diagnosis of ganglioglioma, World Health Organization grade 1 central nervous system tumor was favored after careful review of the clinical, imaging, and microscopic features, including an extensive immunohistochemical staining panel. The chromosomal copy number profile revealed gain/trisomy of chromosome 7, loss/monosomy of chromosome 10, focal homozygous/biallelic deletion of the *CDKN2A* and *CDKN2B* tumor suppressor genes on chromosome 9p21.3, and focal homozygous/biallelic deletion of the *NF1* tumor suppressor gene on chromosome 17q11.2. See Figure 2 for the remaining elements to this case.

spective study of nonenhancing IDH-mutant gliomas also showed progression-free survival improvement in response to a different IDH-mutant inhibitor, ivosidenib.[18] This may ultimately change the current approach of observation and watchful waiting for CNS WHO grade 2 IDH-mutant gliomas. However, the impact of IDH-mutant inhibitors on overall survival is still unknown, and once an IDH-mutant glioma has progressed to higher grade or develops enhancement on magnetic resonance imaging, the therapeutic benefits of IDH-mutant inhibitors are unclear.[19-21] In those settings, RT with adjuvant temozolomide or procarbazine, lomustine, and vincristine is still standard treatment for both IDH-mutant astrocytomas and IDH-mutant oligodendrogliomas,[15,16,22-25] although IDH-mutant inhibitors will certainly be tested in combination with temozolomide and other therapeutics (eg, NCT05609994, NCT05484622, NCT04056910).

Pediatric-Type Diffuse Low-Grade and High-Grade Gliomas

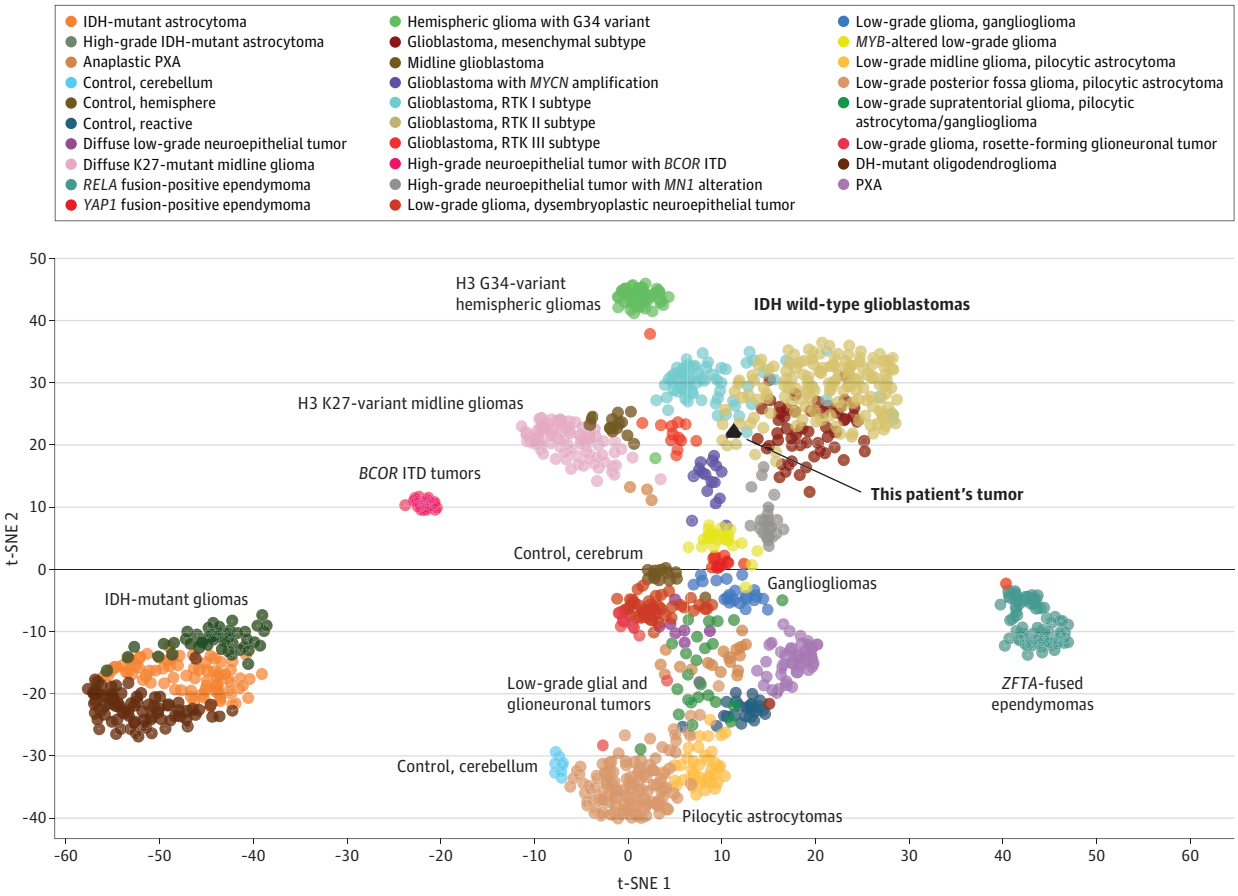
Diffuse gliomas whose molecular profiles are most often seen in children underwent substantial reorganization in the 5th edition of the WHO classification of CNS tumors. Pediatric-type gliomas are now classed separately from adult-type diffuse gliomas and are further subdivided into low grade and high grade. Diffuse low-grade pediatric gliomas include diffuse *MYB*- or *MYBL1*-altered astrocytoma, angiocentric glioma, polymorphous low-grade neuroepithelial tumor of the young (PLNTY), and diffuse MAPK pathway–altered low-grade glioma. These tumors are called diffuse because of an infiltrative growth pattern when visualized microscopically. However, in many circumstances, the tumors do not seem to recur or spread after surgery, meaning that the tumor cells are not actively infiltrating throughout the cerebrum. Since accurately predicting such behavior solely by histopathology is nearly impossible in many instances, advanced molecular testing is essential. Relying solely on NGS without methylation profiling risks misdiagnosis in some contexts. For example, diffuse astrocytoma, *MYB*- or *MYBL1* altered has *MYB* or *MYBL1* fusions, yet so do angiocentric gliomas (eFigure 15 in the Supplement). PLNTY can histologically be mistaken for oligodendrogliomas, gangliogliomas, pilocytic astrocytomas, dysembryoplastic neuroepithelial tumors, and PXA. Like *MYB*-driven tumors, PLNTY also features mitogen-activated protein kinase (MAPK)–activating variants, most often involving *BRAF* or *FGFR2*. Diffuse MAPK pathway–altered low-grade glioma, can histologically resemble gliomas in this and other categories and is driven by targetable *BRAF* or *FGFR1* alterations.[26] All these tumors have MAPK pathway activation and lack IDH-mutant or histone H3 variants. Prog-

Figure 2. Requirement of Advanced Molecular Testing to Accurately Classify and Treat Diagnostically Ambiguous Gliomas, Part 2

A Targeted next-generation DNA sequencing panel results

Variant	Transcript ID	Classification	Reads	Variant allele frequency
Trisomy 7, monosomy 10	NA	Pathogenic	NA	NA
CDKN2A, *CDKN2B* homozygous deletion	All	Pathogenic	NA	NA
NF1 homozygous deletion	All	Pathogenic	NA	NA
PTEN p.Y68H	NM_000314.4	Pathogenic	225	40%
TERT c.-124C>T	NM_198253.2	Pathogenic	598	26%

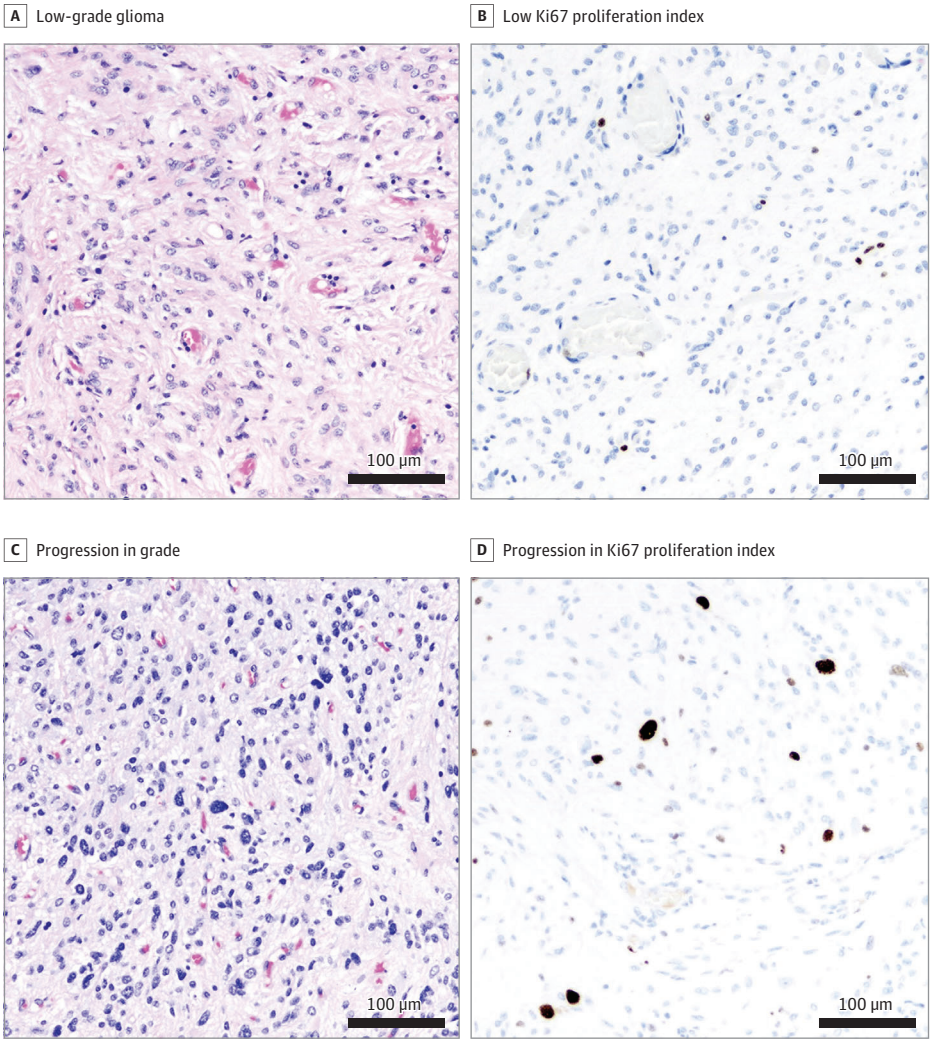
B UMAP dimensionality reduction plot of genome-wide DNA methylation profiles



A woman in her late 40s presented with new onset of aphasia and left arm paresthesia. A, Targeted next-generation DNA sequencing revealed a hotspot substitution variant (c.-124C>T) in the promoter region of the *TERT* gene and a deleterious missense variant (p.Y68H) in the *PTEN* tumor suppressor gene with loss of the remaining wild-type allele, along with the above-mentioned focal deletions involving *CDKN2A/B* and *NF1*. B, Genome-wide DNA methylation interrogation revealed an epigenomic profile that aligned with isocitrate dehydrogenase (IDH) wild-type glioblastoma (mesenchymal subclass). While the histologic features suggested a low-grade glial or glioneuronal neoplasm, such as ganglioglioma, the molecular features indicated an IDH wild-type glioblastoma and led to an accurate final integrated diagnosis of glioblastoma, IDH wild-type, central nervous system World Health Organization grade 4. Despite aggressive external beam radiation and chemotherapy, the patient experienced local disease recurrence at 16 months after initial diagnosis which then demonstrated conventional histologic features of glioblastoma (not shown). ID indicates identifier; ITD, internal tandem duplication; NA, not applicable; PXA, pleomorphic xanthoastrocytoma; RTK, receptor tyrosine kinase; t-SNE, t-distributed stochastic neighbor embedding; UMAP, uniform manifold approximation and projection.

nosis is generally excellent among diffuse pediatric low-grade gliomas, but alterations in cell cycle–associated genes, most notably *CDKN2A/B* homozygous deletion, increase the risk of local regrowth and malignant transformation.[7,26] Thus, even in these low-grade tumors, it is best to screen for genomic copy number variants (CNVs) so higher-risk cases are caught as early as possible. Even though many of these diffuse low-grade tumors do not yet have specific molecularly targeted therapies assigned to them, being able to confidently differentiate them from infiltrative high-grade gliomas using objective molecular testing is a powerful treatment in its own right, as it can provide reassurance to treating physicians and the family as to when observation after surgery is appropriate, sparing

Figure 3. Histone H3 K27M Glioma With Concomitant *BRAF* Alteration



A man in his early 30s developed a mass in the pineal region that was resected. Initial hematoxylin-eosin staining was of a low-grade glioma (A; original magnification ×100) with low Ki67 proliferation index (B; original magnification ×100). Next-generation sequencing showed that the tumor had an *H3-3A* K27M alteration and an activating K601N alteration in *BRAF*. Despite the *H3-3A* variant normally associated with short patient survival, the tumor did not recur until 6 years later, with obvious progression in grade by hematoxylin-eosin staining (C; original magnification ×100) and Ki67 proliferation index (D; original magnification ×100).

young patients from unnecessary toxic treatments. Moreover, the distinction between low-grade and high-grade can be critical in evaluating the need for radiation therapy and/or chemotherapy—an important consideration when taking into account the adverse effects of such adjuvant therapies on the developing brain.[27] The potential therapeutic significance of specific molecular characterization is also highlighted by a recent randomized trial showing that treatment of *BRAF*-variant low-grade gliomas and low-grade neuroglial tumors with dabrafenib and trametinib was superior to conventional chemotherapy, leading to early approval from the US Food and Drug Administration for this molecular-based treatment.[28]

The 5th edition of the WHO classification recognizes 4 high-grade pediatric-type diffuse gliomas: H3 K27–altered diffuse midline glioma; H3 G34–mutant diffuse hemispheric glioma; H3 and IDH wild-type diffuse pediatric-type high-grade glioma (pHGG); and infant-type hemispheric glioma (ITHG). H3 K27-altered diffuse midline glioma arising in the brain stem had long been known as diffuse intrinsic pontine glioma prior to the molecular redefinition of these tumors (and the realization that they can occur in other midline sites outside the brain stem). Whereas K27 alterations in the histone-encoding *H3-3A* or other histone H3 genes tend to arise in the midline, those with G34 variants in the same gene arise peripherally in the cerebral hemispheres.[29] While both tumors show loss of histone trimethylation and are mutually exclusive with IDH-mutant tumors, K27-mutant midline gliomas virtually never have *MGMT* promoter methylation, whereas G34-mutant gliomas usually do.[29,30] Adding to the complexity of these histone-driven gliomas, some K27-altered midline gliomas also contain activating *BRAF* or *FGFR1* variants. Such tumors are radiologically more nodular than, and are epigenomically distinct from, most K27-altered midline gliomas and are associated with longer patient survival (**Figure 3**).[31] pHGG may strongly resemble adult-type glioblastoma or undifferentiated, embryonal-like tumors and often contains genetic alterations most commonly found in IDH wild-type glioblastoma, like those involving *PDGFRA*, *MET*, *MYCN*, or *NF1*, although pHGG have a distinct genomic DNA methylation pattern.[26] ITHG are not equivalent to desmoplastic infantile ganglioglioma/astrocytoma, even though they occur in the same age range and may resemble each other microscopically. Accurately diagnosing ITHG by molecular testing is critical because these tumors are driven solely by receptor tyrosine kinase (RTK)–

activating fusions. As a result, ITHG are highly responsive to targeted inhibition of whichever RTK is activated, most often *NTRK1-3*, *ALK*, or *ROS1*.[32]

It bears re-emphasizing that adult-type and pediatric-type gliomas indicate that there is no clear-cut age boundary at which these tumors do or do not develop. For example, adult-type IDH-mutant gliomas can occur in children, and pediatric-type gliomas (either H3-mutant or H3 wild-type) can arise in adults, even those of advanced age. Standard histopathology with immunohistochemistry is somewhat helpful but often cannot distinguish these tumor types from each other. Therefore, routine broad-based multidimensional molecular testing of all gliomas in all patients is the best way to ensure that these tumors are properly diagnosed. In one study of more than 1200 pediatric CNS tumors, multiomic profiling enhanced diagnostic value in more than one-half of cases, including more tumor subtype precision, identification of therapeutic targets, and/or detection of cancer predisposition syndromes.[33]

Circumscribed Astrocytic Gliomas

Circumscribed astrocytic glioma is an entirely new category in the WHO classification system. It is mostly composed of tumors that had long been recognized, including pilocytic astrocytoma, PXA, subependymal giant cell astrocytoma, and chordoid glioma, all of which tend to have relatively sharp tumor-nontumor boundaries. As with diffuse pediatric-type low-grade gliomas, advanced molecular diagnostics reliably differentiate these tumors from infiltrative gliomas, thus substantially altering patient prognosis and overall treatment strategy. For example, it is very common for PXA to be mistaken for other high-grade gliomas, especially IDH wild-type glioblastoma, when relying only on microscopy (**Figure 4**A and B). But patients with PXA have a median 5-year survival rate of 60% to 90%, whereas less than 10% of patients with IDH wild-type glioblastoma survive that long.[26] Because most PXAs contain *BRAF* V600E, RAF and MEK inhibitor combinations can be very effective in those tumors.[34,35] Likewise, detecting a *BRAF* fusion not only suggests a pilocytic astrocytoma but also raises the possibility of treatment with RAF and/or MEK inhibitors.[36,37] Prospective randomized international trials are currently underway comparing MEK-inhibitor therapy with conventional chemotherapy for newly diagnosed, progressive *BRAF*-fusion low-grade gliomas. Since subependymal giant cell astrocytoma is characterized by mTOR-activating variants, detecting such a variants not only confirms the diagnosis but also provides an Food and Drug Administration–approved indication for treating with an mTOR inhibitor.[38] As an example of specific molecular alteration(s) now being embedded in a diagnosis, *MN1*-altered astroblastoma tumors can no longer be diagnosed as astroblastomas unless they contain a rearrangement involving either the *MN1* or *BEND2* genes.[26] This is because astroblastomas are very rare and can histologically resemble a lot of other gliomas that have divergent outcomes, including ependymomas, glioblastomas, PXA, or embryonal tumors.[26] High-grade astrocytoma with piloid features (HGAP) contains *BRAF* abnormalities or *NF1* inactivation, along with *ATRX* alterations and *CDKN2A/B* deletion.[39] DNA methylation profiling is currently considered by the WHO classification as the only reliable way to diagnose HGAP, which can resemble pilocytic astrocytoma or IDH wild-type glioblastoma even though it has worse survival than the former and better survival than the latter.[39]

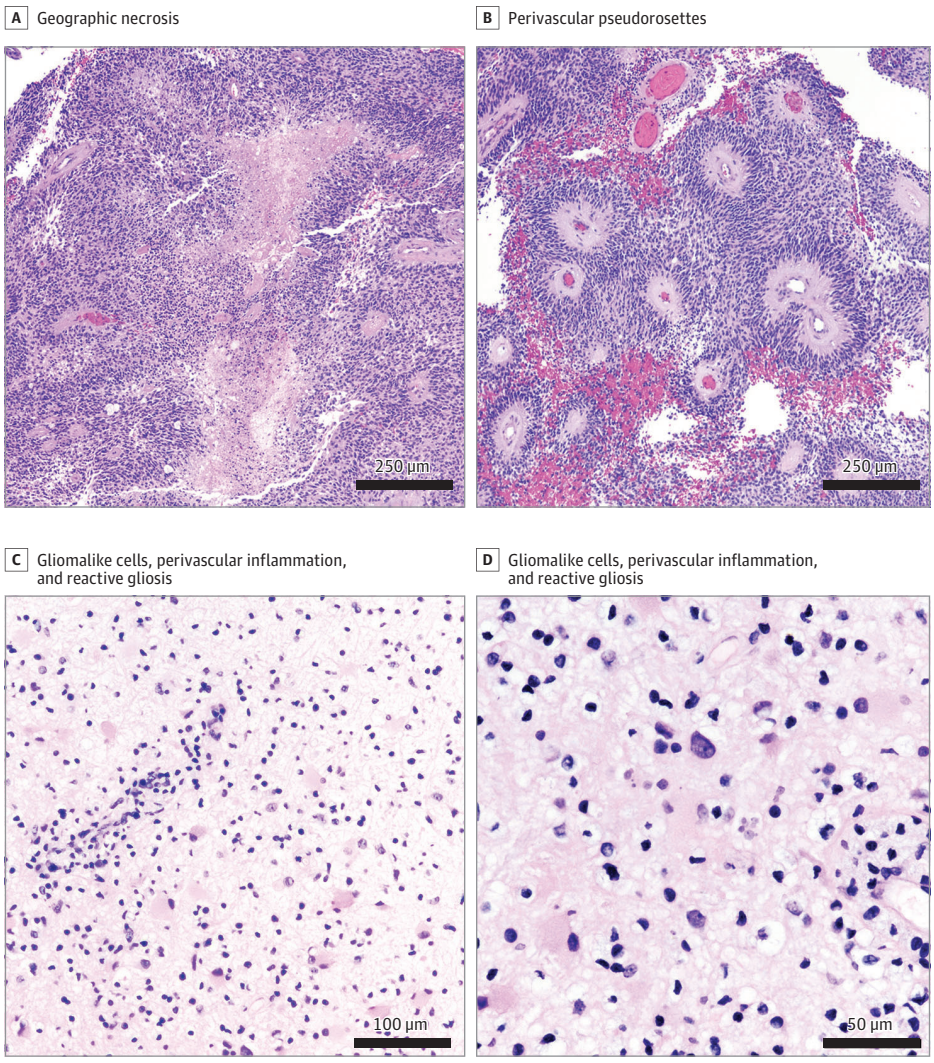
Glioneuronal and Neuronal Tumors

The 5th edition of the WHO classification added 3 new types of glioneuronal and neuronal tumors: diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters, featuring chromosome 14 monosomy; myxoid glioneuronal tumor, with K385L or K385I variants in *PDGFRA*; and multinodular and vacuolating neuronal tumor, containing a MAPK pathway–activating variant, most commonly involving *MAP2K1*. All the preexisting tumors in this subgroup are also characterized by specific molecular alterations (Table) and can be differentiated from each other and from higher-grade mimickers by DNA methylation profiling. While all these glioneuronal/neuronal tumors are fairly indolent, they may look like higher-grade tumors, eg, dysembryoplastic neuroepithelial tumor resembling infiltrating oligodendroglioma, or ganglioglioma vs the infiltrative edge of a glioblastoma. Immunohistochemistry is not sensitive or specific enough to reliably make these distinctions. Glioneuronal/neuronal tumors can even resemble focal cortical dysplasia in their clinical presentation, location, and histopathology—indeed, the line between focal cortical dysplasia and a CNS WHO grade 1 glioneuronal/neuronal tumor is rather blurry.[40] A related salient point is that because the molecular features of low-grade and high-grade CNS neoplasms have been so thoroughly characterized, the complete absence of any of those features can be extremely helpful in ruling out a neoplasm that requires adjuvant therapy or extensive postsurgical monitoring (Figure 4C and D).

Ependymal Tumors

In the 5th edition of the WHO classification, most ependymoma subtypes now include specific molecular alterations in their names (Table),[41] such as fusions involving either *ZFTA* or *YAP1* in supratentorial tumors. Not only is detection of those fusions useful in differentiating supratentorial ependymomas from *FOXR2*-driven or *BCOR*-driven embryonal tumors but also from *MN1*-altered astroblastoma.[7,26] Furthermore, *ZFTA* fusion–positive ependymomas may be more aggressive than *YAP1* fusion–positive ependymomas.[26] In the posterior fossa, DNA methylation profiling is the definitive way to differentiate between posterior fossa A and posterior fossa B tumors. This is important because, while both are difficult to completely excise, posterior fossa B ependymomas are less aggressive and are more treatable, even at recurrence.[42,43] Posterior fossa ependymal tumors also include those in the subependymoma or mixed ependymoma-subependymoma category. Screening posterior fossa ependymal tumors for *TERT* promoter variants, chromosome 6q loss, and chromosome 1q gain are also useful in planning adjuvant therapy and frequency of ependymoma monitoring by magnetic resonance imaging.[44-46] While spinal ependymomas are overall more benign than supratentorial or posterior fossa ependymomas, those with *MYC* or *MYCN* amplification act more malignant, including rapid cerebrospinal fluid dissemination.[47] Myxopapillary ependymomas have their own unique DNA methylation profile, and because they can grow back and spread around the spinal cord, they have been upgraded from

Figure 4. Examples of Advanced Molecular Testing Enhancing Patient Care



A man in his mid-50s had a right-sided frontal parietal mass that, by histopathology using hematoxylin-eosin staining, had abundant geographic necrosis (A; original magnification ×40) and apparent perivascular pseudorosettes (B; original magnification ×40). Isocitrate dehydrogenase wild-type glioblastoma and grade 3 ependymoma were in the original differential diagnosis, but upon revisiting the case years later, next-generation sequencing detected a *BRAF* V600E variant and matched to pleomorphic xanthoastrocytoma by methylation profiling. Seven years after initial surgery, the tumor still had not recurred or spread elsewhere in the central nervous system. In another case, a woman in her mid-20s had a history of seizures since childhood that recently became uncontrollable with antiepileptic drugs. An ill-defined right-sided parietal mass was biopsied, and on hematoxylin-eosin staining, there were some gliomalike cells, perivascular inflammation, and reactive gliosis (C [original magnification ×100] and D [original magnification ×200]), but no mitoses, necrosis, or microvascular proliferation. The only abnormality detected by next-generation sequencing, copy number variant array, methylation profiling, and fusion screening was copy-neutral loss of heterozygosity involving 5q21.1-5q21.3. Thus, the final diagnosis was low-grade glioma vs glial-glioneuronal malformation, with the recommendation of watchful waiting and withholding adjuvant therapy until the lesion recurred.

CNS WHO grade 1 to grade 2.[48] Subependymomas, in contrast, remain CNS WHO grade 1. However, these tumors can histologically resemble CNS WHO grade 2 ependymomas, so identifying them by molecular testing, including screening for fusions, may avoid unnecessary postsurgical radiation.

CNS Embryonal Tumors

By far the most common embryonal tumor in the CNS is medulloblastoma, which is split into 4 main molecular-driven and outcome-driven subtypes: WNT activated; SHH activated, *TP53* wild-type; SHH activated, *TP53* variant; and non-WNT, non-SHH tumors (Table). SHH-activated medulloblastomas with *TP53* variants are much more aggressive than those without *TP53* variants,[49] and WNT-activated medulloblastomas are highly responsive to adjuvant therapy, especially when arising in children.[50,51] As a result, clinical trials are testing lower doses of craniospinal RT and less intensive chemotherapy regimens in pediatric patients with WNT-activated medulloblastoma. Among the non-WNT, non-SHH medulloblastomas, it appears that there are actually at least 8 different methylation patterns that vary in outcome, with subgroups 6 and 7 being the best and subgroups 2 and 3 being the worst.[52] *MYC* (and possibly

MYCN) amplification is also an adverse prognostic marker.[26] Based on such molecular-based classifications, international cooperative group trials are now being formatted not only for the conventionally stratified groups of children with average-risk and high-risk disease but also for the study of more intensive treatment approaches in very high-risk patients. The other embryonal tumors in the 5th edition are extremely rare and are difficult to differentiate from each other histologically; hence, the heavy reliance on specific genomic alterations as in *FOXR2*-activated CNS neuroblastoma, CNS tumor with *BCOR* internal tandem duplication, and *SMARCB1* alterations in Cribriform neuroepithelial tumor.[7,26] CNS tumor with *BCOR* internal tandem duplication can look microscopically like ependymoma or glioblastoma and has a wide range of possible clinical outcomes.[53] Cribriform neuroepithelial tumor resembles choroid plexus carcinoma or atypical teratoid-rhabdoid tumor, although patients with cribriform neuroepithelial tumor have much better response to therapy and longer overall survival than either choroid plexus carcinoma or atypical teratoid-rhabdoid tumor.[54] Prior to advanced molecular diagnostics, these tumors usually ended up being called *primitive neuroectodermal tumors*. However, since molecular testing nearly always reassigns a primitive neuroectodermal tumors into something else, the term is no longer used.[55] Because embryonal tumors like those driven by *BCOR* and *FOXR2* are so rare, focused clinical trials will need to be done on a national or international scale.[56,57]

Meningiomas

Like other CNS tumors, meningiomas tend to have specific variants according to certain morphologic subtypes or locations (eg, *SMARCE1* variants in clear cell meningiomas, *SMO* variants in olfactory groove meningiomas) (Table). Detection of such variants can help refine the meningioma subtype, but only *TERT* promoter alteration and/or homozygous *CDKN2A/B* deletion officially confer a CNS WHO grade 3 diagnosis irrespective of histopathology.[7,26,58,59] However, emerging research consistently shows that meningioma prognosis is driven by larger chromosomal losses, like those involving 1p, and that genomic copy number profiling more accurately stratifies patient outcomes when integrated with histopathology, methylation profiling, *TERT* promoter screening, and *CDKN2A/B* status.[58,60-64] This greatly affects clinical management, because patients with CNS WHO grade 2 meningioma undergoing gross total resection may or may not receive adjuvant RT, while incompletely resected CNS WHO grade 2 tumors and all CNS WHO grade 3 tumors require postoperative RT.[65] Integrating molecular data can also identify histologic CNS WHO grade 2 meningiomas that are more likely to be indolent, thus avoiding unnecessary RT.[60]

Craniopharyngiomas

Craniopharyngiomas arising in the sella come in 2 histotypes: adamantinomatous and papillary. The former have variants in the *CTNNB1* gene, encoding β-catenin and resulting in constitutive activation of the WNT signaling pathway.[66] The latter contain *BRAF* V600E, which renders them highly responsive to the combination of BRAF and MEK inhibitors.[67] Differentiating between the 2 subtypes, and proving the presence of *BRAF* V600E in papillary craniopharyngiomas, is therefore important when trying to mitigate disease recurrence and guide management.

Advanced Molecular Biomarker Testing Relative to the Total Cost of CNS Tumor Patient Care

The combined cost of NGS for alteration and fusion screening and arrays for genomic copy number and DNA methylation profiling costs between $5000 and $10 000. These complementary assays synergize to provide clinically vital information. Furthermore, comprehensive molecular testing incorporating NGS, CNV, and genomic DNA methylation provides better diagnostic and treatment-relevant information at a lower cost than the sum of all the individual single-gene tests that would otherwise need to be performed. Especially as more therapies are developed for specific molecular alterations (eg, *BRAF*, *NTRK*, *FGFR3*) that cut across multiple types of tumors and are approved for targeted treatments independent of histology (eFigure 16 in the Supplement),[34,35,68,69] it is essential to have platforms that screen for all actionable alterations simultaneously. Similarly, screening gliomas for DNA mismatch repair deficiency identifies pediatric and adult patients who might benefit from immunotherapy.[70,71] NGS, CNV array, and genomic methylation profiling can all be done using only 4 cm^2 of 5 µm–thick tissue (approximately 15 unstained slides) from the best block and can all be completed within 2 to 3 weeks.

Molecular screening of CNS neoplasms greatly reduces the likelihood of a misdiagnosis and focuses subsequent expensive follow-up imaging and treatments on those patients who are most likely to benefit. Neurosurgical tumor biopsy and/or resection costs up to $100 000 or more.[72] In the case of an equivocal or indeterminate biopsy, molecular testing can inform an accurate diagnosis even when the histopathologic features are not definitive. Adjuvant therapy costs up to $200 000 for radiation therapy, up to $50 000 per gamma knife treatment, $2000 to $5000 per month for chemotherapy, $20 000 per month for tumor-treating fields, and $30 000 per month for bevacizumab.[72-74] Given all this, the Brain Tumor Foundation estimates that total costs of brain tumor patient care, including rehabilitation and hospice, approach $800 000. Thus, even a full set of advanced molecular tests is less than 2% of total health care expenditures for patients with brain tumors and that 2% has an outsized impact on whether and how the other 98% is spent.

Conclusions

In summary, the new WHO classification and NCCN guidelines are designed to provide patients with CNS tumors with the best possible outcomes. But these guidelines are only as impactful as the extent to which they are used. We therefore urge health insurance payers, both public and private, to update their coverage policies accordingly.

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