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The next evolution of neuro-oncology testing

Clarify diagnosis with precise tumor classification

Guideline-aligned testing at the right time

Identifying and classifying molecular markers present in central nervous system (CNS) tumors can be a complex challenge. 2021 World Health Organization Classification of Tumors of the CNS, 5th edition (WHO CNS5) guidelines indicate four key molecular biomarkers for histo-molecular diagnosis: DNA mutations, RNA fusions when appropriate, copy number abnormalities, and DNA methylation profile.

Integrated histology + molecular diagnosis (2021)

WHO CNS5 requires a **histo-molecular synthesis** for most CNS tumors. **~78%** of tumor entities require at least one **essential molecular marker** for accurate diagnosis or grading.

The addition of methylation profiling is especially critical for patients with CNS tumors, as 1 in 5 CNS tumors lack sufficient histology/IHC evidence to be definitively diagnosed. Methylation profiling complements next-generation sequencing (NGS) and chromosomal microarray testing, allowing physicians to classify the tumor subtype based on its unique signature to resolve challenging or conflicting cases.

- **Integrated diagnosis** replaces histology-only classification.
- **Molecular criteria** can define tumor type and WHO grade.
- **DNA, copy number variant (CNV), and methylation** are core diagnostic tools.

A testing workflow that integrates methylation profiling, as described in the diagram and case study on the following pages, enables accurate diagnoses and confident treatment decisions.

Integrated neuro-oncology testing aligned with clinical guidelines

Start with triage	First-line molecular choice	Cytogenetics/CNV	Genome-wide methylation	Integrate all results
Histology + site + age Assess tissue quantity and purity	Specific DNA mutations/ RNA fusions question → use NGS	Use for hallmark gains, losses, amplifications, deletions, and loss of heterozygosity	Broad differential → consider methylation	Final diagnosis should combine clinical, radiology, histology, DNA, CNV, and methylation findings

Patient case study: How methylation clarified a challenging tumor

A 46-year-old man presents with a right cerebellar enhancing tumor

Initial workup	**Histology:** Mitotically active diffuse astrocytic glioma with microvascular proliferation.
	Immunochemistry: • H3 K27M and *IDH1*-R132H: Negative • H3 K27me3: Normal preserved expression • *ATRX*: Abnormal loss of expression • P53: Variable expression
	Molecular testing: • *NF1/ATRX* mutations but no *IDH1/IDH2/TERT* mutations • *CDKN2A/B* homozygous deletion but no +7/–10 signature or *EGFR* amplification
Clinical challenge	**Inconclusive findings:** Tumor suspected to represent high-grade astrocytoma with piloid features (HGAP), which is only diagnosable by methylation profiling.
Intervention	Genome-Wide DNA Methylation Profiling (Mayo ID: MTNON)

Result	**Refined molecular diagnosis:**
	• HGAP
	  • Supporting features: *NF1/ATRX* mutations
	  • *CDKN2A/B* homozygous deletion

Outcome	DNA methylation profiling enabled a definitive diagnosis. • Definitive tumor classification aligned with WHO framework. • Improved prognostic confidence (available data suggests intermediate CNS grade 3 behavior). • Avoiding misclassification and inappropriate treatment.

Primary central nervous system tumor genetic testing ordering guide*

TUMOR GROUP	TESTING TO ESTABLISH DIAGNOSIS	DIAGNOSTIC SUPPORT/ CONFIRMATION TESTING
High-grade gliomas	Order all of the following: • **NONCP / Neuro-Oncology Expanded Gene Panel with Rearrangement, Tumor** • **MTNON / Neuro-Oncology Genome-Wide Methylation Array Analysis, Tumor** especially if tumors such as high-grade astrocytoma with piloid features or diffuse pediatric-type high-grade glioma, H3-wildtype, and IDH-wildtype are possible	Consider **CMAPT / Chromosomal Microarray, Tumor, Formalin-Fixed Paraffin-Embedded**
Lower-grade gliomas	Order the following: • **NONCP** • **CMAPT**	Consider **MTNON** and/or **CMAPT**
Ependymoma and ependymal-like tumors	Order **MTNON**	Consider **NONCP** and/or **CMAPT**
Medulloblastoma and other embryonal tumors	Order **MTNON**	Consider **NONCP**
Meningioma	Order the following: • **NONCP** • **CMAPT**	Consider **MTNON**
Other tumor types	Consider the following, based on the differential diagnosis: • **NONCP** • **CMAPT** • **MTNON**	
Diagnostically challenging cases	Order all of the following: • **NONCP** • **CMAPT** • **MTNON** Can be especially helpful in high-grade pediatric brain tumors and tumors with unusual clinical, radiologic, and/or morphologic features.	

*Genetic testing results should be correlated with histopathological and clinico-radiological findings. Prioritize NONCP (next-generation sequencing: NGS) when a specific mutation/fusion needs to be confirmed, and NONCP and/or CMAPT (copy number variant: CNV) analysis when tumor purity and/or amount is low (<60% tumor, <144 mm^2 tissue).

Mayo Clinic Laboratories neuro-oncology testing

Mayo Clinic Laboratories offers testing that was developed by our physicians and laboratorians to support the Mayo Clinic practice, in alignment with WHO guidelines, as described on previous pages.

21%

of CNS tumor diagnoses were changed or refined with methylation profiling[1]

14%

of CNS tumor cases were identified as being diagnostically mismatched through methylation profiling[1]

7%

of CNS tumor cases received additional prognostic insight through methylation profiling[1]

Methylation profiling

Methylation profiling complements DNA NGS and microarray testing and is essential for accurate diagnosis.

Our first-in-class, clinical genome-wide DNA methylation array (Mayo ID: MTNON) provides the methylation profile and MGMT promoter methylation status of CNS tumors through the use of the Illumina Infinium Methylation EPIC v2.0 combined with the National Institutes of Health-developed, AI-powered CNS tumor classifier algorithm v.2.0, and the Mayo Clinic-developed nearest-neighbors assisted unsupervised analysis (NN method).

MTNON | Neuro-Oncology Genome-Wide Methylation Array Analysis

- Provides methylation profile and MGMT promoter methylation status in primary tumors and a subset of metastatic CNS tumors.
- Aligns with integrated diagnosis recommendations from the 2021 WHO Classification of CNS Tumors.
- Enables definitive diagnosis of epigenetically defined 2021 CNS tumor types, such as high-grade astrocytoma with piloid features.
- Provides clinically actionable subclassification of key CNS tumor types, such as ependymoma, medulloblastoma, and diffuse pediatric-type high-grade gliomas.
- Clarifies ambiguous diagnosis and reduces diagnostic uncertainty in cases where sequencing and chromosomal microarray results are inconclusive.
- Reduces tumor misclassification, which could lead to inappropriate therapy.
- Results include a level of confidence for the predicted classifications, providing diagnostic clarity.

Next-generation sequencing

Our next-generation sequencing (NGS) panel is part of our MayoComplete suite of NGS panels for hematologic and oncologic conditions. This comprehensive panel evaluates for the presence of somatic mutations in 89 genes and gene fusions in 1,445 genes, including most abnormalities described by the 2021 WHO classifications.[2]

NONCP | Neuro-Oncology Expanded Gene Panel with Rearrangement

- Simultaneously tests for multiple 2021 WHO diagnostic/grading molecular biomarkers, including mutations and fusions.
- Includes abnormalities described in adult-type and pediatric-type brain tumors.
- Offers increased sensitivity to detect lower-level tumor purity. Includes 89 clinically relevant DNA genes, and detection of gene fusions and transcript variants in 1,445 RNA genes.

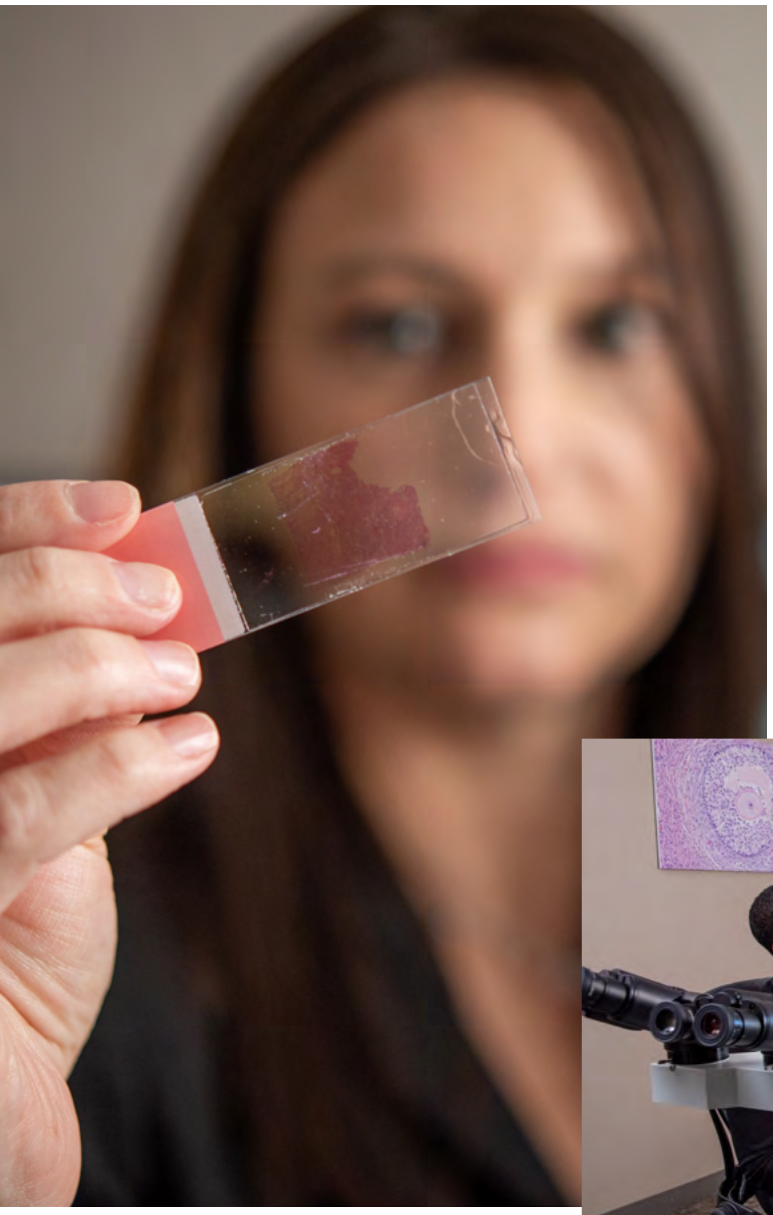
Chromosomal microarray

Chromosomal microarray provides high-resolution assessment of copy number variants across the genome. According to published testing guidelines, when the abnormalities detected in our assay are found in diffuse astrocytic gliomas, IDH-wildtype, the tumor should be considered as having molecular features of glioblastoma.[3]

CMAPT | Chromosomal Microarray

- Superior analysis of the 1p/19q co-deletion in gliomas.
- Detects abnormalities, including the gain of chromosome 7, loss of chromosome 10, and *EGFR* amplification.
- Simultaneously tests for multiple 2021 WHO diagnostic biomarkers, including copy number variants and amplifications.

Pathology consultations



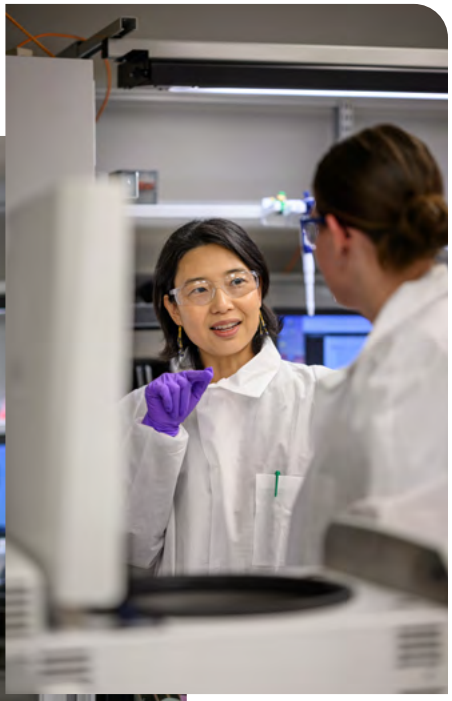
For many pathologists and community hospitals, less than 5% of their surgical pathology cases include patients with brain tumors. Our second-opinion consultations enable access to Mayo Clinic neuropathologists who can help choose the most appropriate molecular and cytogenetic testing. Our experts help integrate laboratory results with clinical histopathological information for a comprehensive report with patient-specific diagnostic, prognostic, and therapeutic information.

PATHC | Pathology Consultation

- Offers expert guidance to assist with rare and complex cases.

Learn more about our comprehensive approach to neuro-oncology testing

Mayo Clinic Laboratories' team is made up of neuropathologists, oncologists, and geneticists specializing in immunohistochemical, molecular, and cytogenetic diagnostic testing focused on patient outcomes. Our integrated clinical, genetic, and laboratory testing experts are available 24/7 to provide expertise and consultative support, removing the guesswork from test selection and results interpretation.



The classifier used in this assay was developed by **Kenneth Aldape, M.D.**, a neuropathologist with molecular pathology expertise and researcher in the Genetics and Genomics Laboratory at Mayo Clinic, when he was the head of the Department of Pathology at the National Cancer Institute. **Cristiane Ida, M.D.**, is a Mayo Clinic molecular pathologist with neuropathology expertise and researcher in Mayo Clinic's Genetics and Genomics Laboratory.

References

[1]Galbraith K, Vasudevaraja V, Serrano J, et al. Clinical utility of whole-genome DNA methylation profiling as a primary molecular diagnostic assay for central nervous system tumors-A prospective study and guidelines for clinical testing. *Neurooncol Adv*. 2023 Jun 26;5(1):vdad076. doi:10.1093/noajnl/vdad076. Erratum in: *Neurooncol Adv.* 2023 Sep 23;5(1):vdad114. doi:10.1093/noajnl/vdad114. PMID: 37476329; PMCID: PMC10355794. [2]Siddaway R, Glembocki AI, Arnoldo A, et al. Clinical utility of targeted RNA sequencing in cancer molecular diagnostics. *Nat Med*. 2025 Oct;31(10):3524-3533. doi: 10.1038/s41591-025-03848-8. Epub 2025 Jul 17. PMID: 40676318. [3]WHO CNS5: 2021 WHO Classification of Tumors of the Central Nervous System.

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