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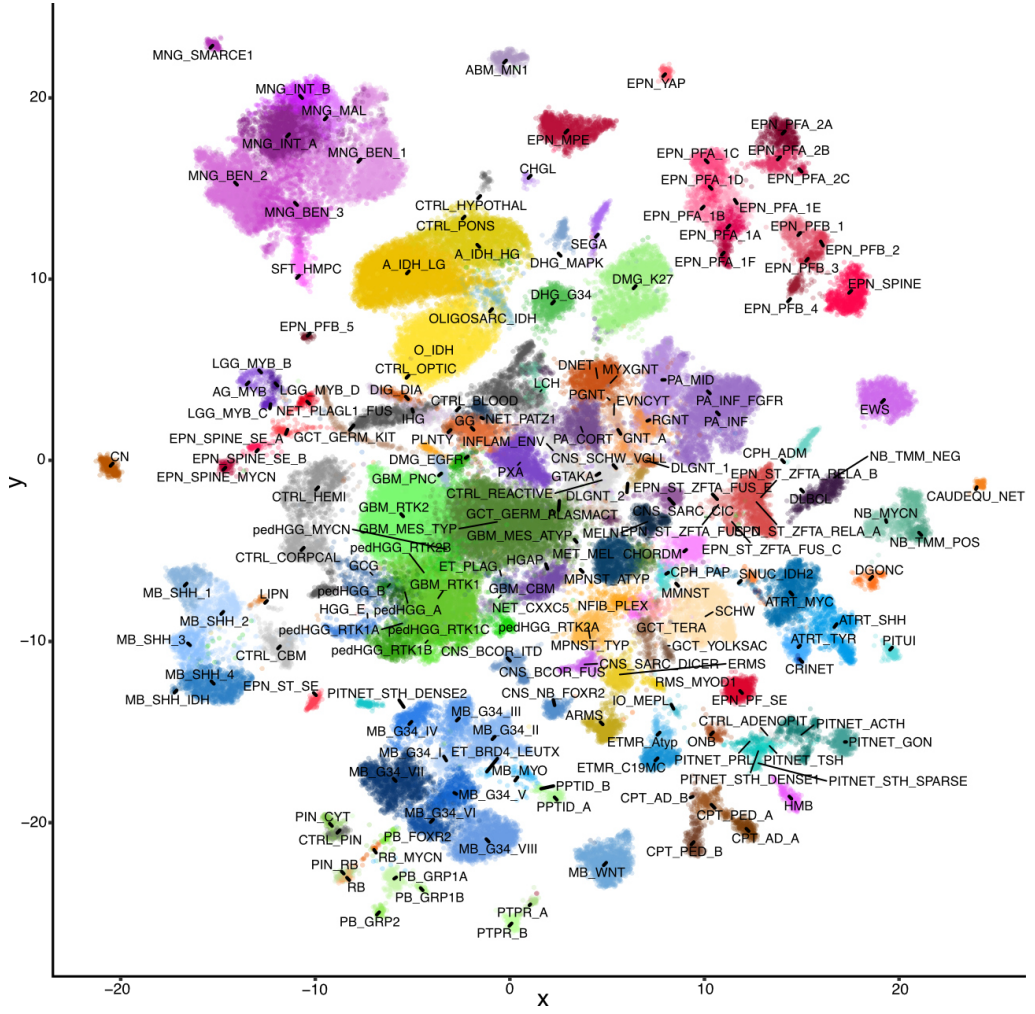
Molecular Tumor Classification - Revolutionizing Precision Oncology



https://epignostix.com

AI-powered models for cancer diagnostics

- **Diagnostic AI-powered models** assign patient samples precisely and objectively to cancer classes to guide optimal patient treatment
- Cancer tissue biopsy samples are subjected to analysis in **molecular pathology.** DNA methylation molecular signatures are our core technology, we are expanding into cancer biomarkers and digital pathology

More than **180,000 patient samples** analyzed to date demonstrate rapid adoption and a high unmet clinical need

Classifiers are locked machine learning models

- DNA-methylation signatures provide a unique **fingerprint** of cancer cells.
- Classifiers are locked machine learning models that apply a fixed algorithm. The software does not employ continuous learning, adaptive AI, or real-time model updates. It uses a predefined set of classification rules derived from a validated training dataset.
- Classifiers are trained on a curated dataset using supervised machine learning techniques. The model parameters remain unchanged after deployment, ensuring consistent and reproducible classification performance across all users.
- The output report offers highly **robust, objective diagnoses** with a well-calibrated confidence score. The large validation cohort enables **reliable diagnosis of rare cancer subtypes.**

Methylation-data is generated from tissue biopsy

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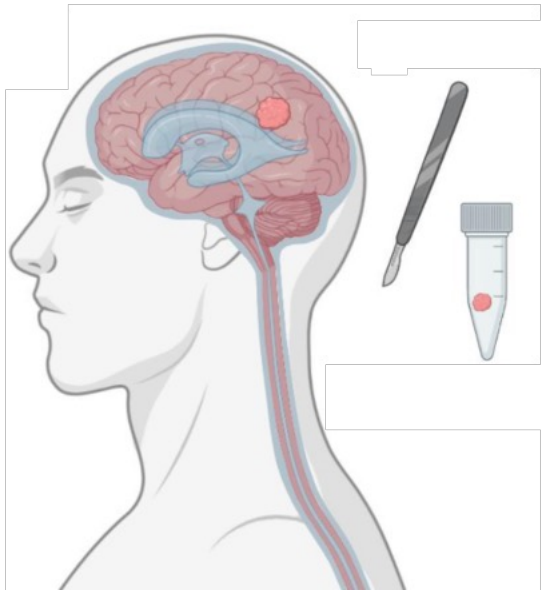
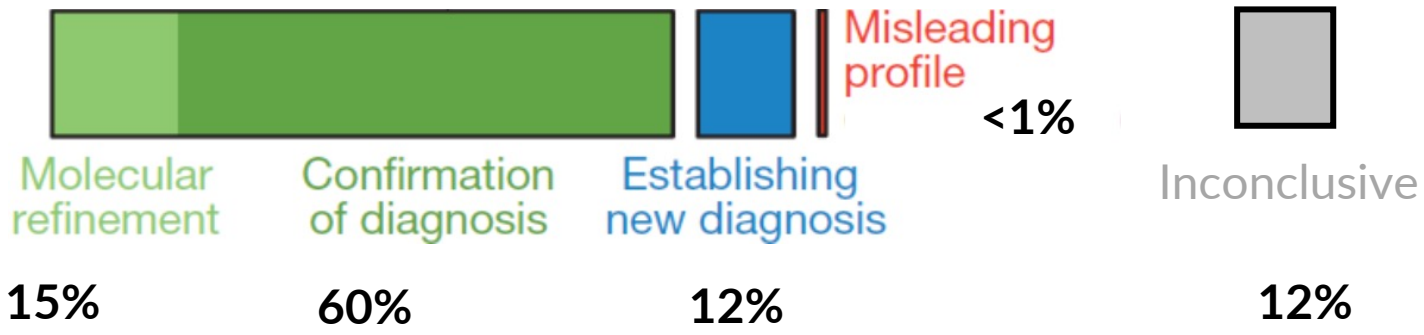
Tissue Biopsy → DNA Extraction → Methylation Data → Classifier Analysis → Individual Patient Management Plan

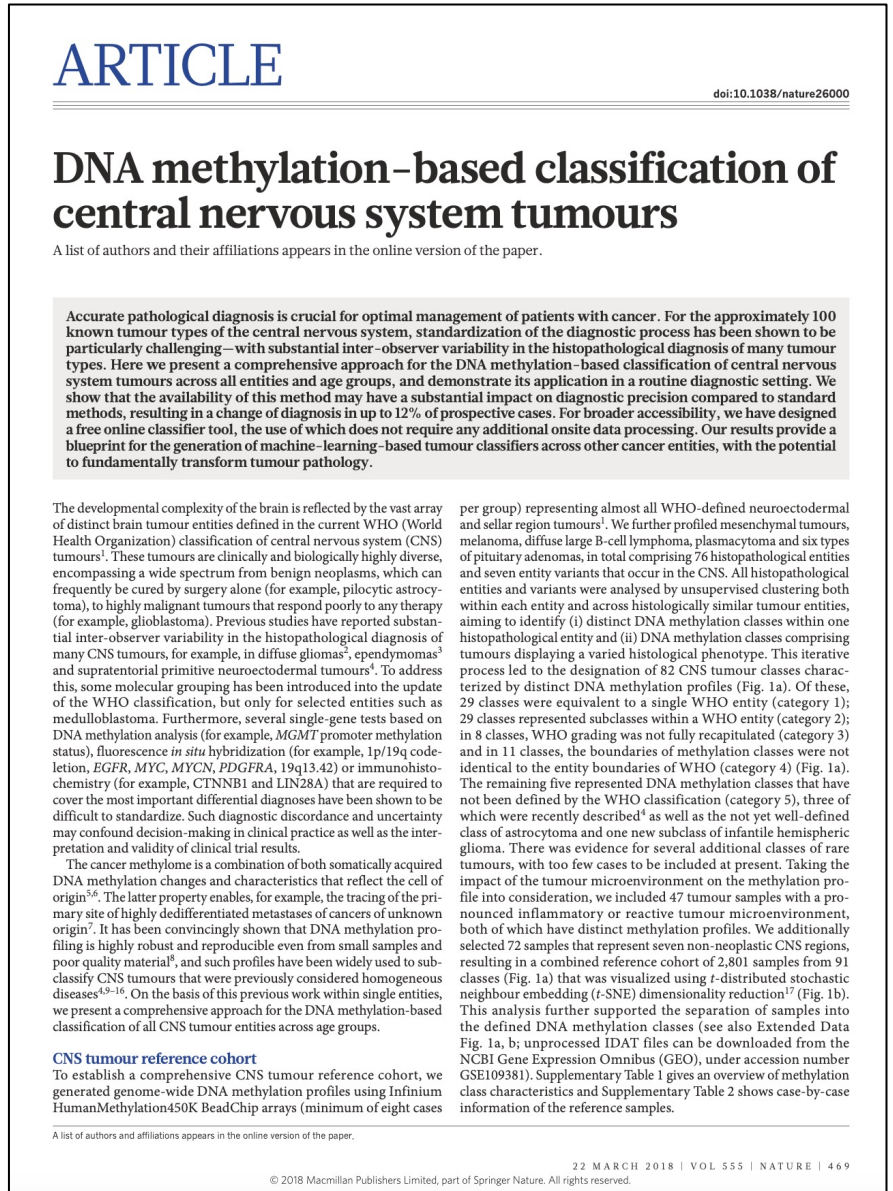


Clinical utility demonstrated in broad body of evidence

- A detailed prospective study of approximately 1,000 brain cancers revealed in 27% of cases a **change or refinement in diagnosis compared with the current standard-of-care**



- Classification enables the selection of the most appropriate treatment from established treatment options
- Precise diagnostics will improve clinical management, benefiting healthcare providers, healthcare professionals and the patients & families



ARTICLE

doi:10.1038/nature26000

DNA methylation-based classification of central nervous system tumours

A list of authors and their affiliations appears in the online version of the paper.

Accurate pathological diagnosis is crucial for optimal management of patients with cancer. For the approximately 100 known tumour types of the central nervous system, standardization of the diagnostic process has been shown to be particularly challenging—with substantial inter-observer variability in the histopathological diagnosis of many tumour types. Here we present a comprehensive approach for the DNA methylation-based classification of central nervous system tumours across all entities and age groups, and demonstrate its application in a routine diagnostic setting. We show that the availability of this method may have a substantial impact on diagnostic precision compared to standard methods, resulting in a change of diagnosis in up to 12% of prospective cases. For broader accessibility, we have designed a free online classifier tool, the use of which does not require any additional onsite data processing. Our results provide a blueprint for the generation of machine-learning-based tumour classifiers across other cancer entities, with the potential to fundamentally transform tumour pathology.

The developmental complexity of the brain is reflected by the vast array of distinct brain tumour entities defined in the current WHO (World Health Organization) classification of central nervous system (CNS) tumours[1]. These tumours are clinically and biologically highly diverse, encompassing a wide spectrum from benign neoplasms, which can frequently be cured by surgery alone (for example, pilocytic astrocytoma), to highly malignant tumours that respond poorly to any therapy (for example, glioblastoma). Previous studies have reported substantial inter-observer variability in the histopathological diagnosis of many CNS tumours, for example, in diffuse gliomas[2], ependymomas[3] and supratentorial primitive neuroectodermal tumours[4]. To address this, some molecular grouping has been introduced into the update of the WHO classification, but only for selected entities such as medulloblastoma. Furthermore, several single-gene tests based on DNA methylation analysis (for example, *MGMT* promoter methylation status), fluorescence *in situ* hybridization (for example, 1p/19q codeletion, *EGFR*, *MYC*, *MYCN*, *PDGFRA*, 19q13.42) or immunohistochemistry (for example, CTNNB1 and LIN28A) that are required to cover the most important differential diagnoses have been shown to be difficult to standardize. Such diagnostic discordance and uncertainty may confound decision-making in clinical practice as well as the interpretation and validity of clinical trial results.

The cancer methylome is a combination of both somatically acquired DNA methylation changes and characteristics that reflect the cell of origin[5,6]. The latter property enables, for example, the tracing of the primary site of highly dedifferentiated metastases of cancers of unknown origin[7]. It has been convincingly shown that DNA methylation profiling is highly robust and reproducible even from small samples and poor quality material[8], and such profiles have been widely used to subclassify CNS tumours that were previously considered homogeneous diseases[4,9–16]. On the basis of this previous work within single entities, we present a comprehensive approach for the DNA methylation-based classification of all CNS tumour entities across age groups.

CNS tumour reference cohort

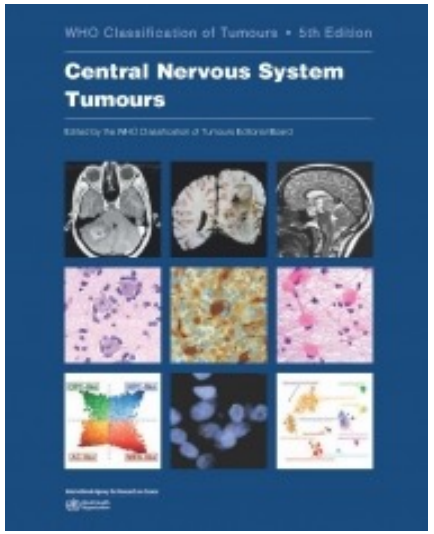
To establish a comprehensive CNS tumour reference cohort, we generated genome-wide DNA methylation profiles using Infinium HumanMethylation450K BeadChip arrays (minimum of eight cases per group) representing almost all WHO-defined neuroectodermal and sellar region tumours[1]. We further profiled mesenchymal tumours, melanoma, diffuse large B-cell lymphoma, plasmacytoma and six types of pituitary adenomas, in total comprising 76 histopathological entities and seven entity variants that occur in the CNS. All histopathological entities and variants were analysed by unsupervised clustering both within each entity and across histologically similar tumour entities, aiming to identify (i) distinct DNA methylation classes within one histopathological entity and (ii) DNA methylation classes comprising tumours displaying a varied histological phenotype. This iterative process led to the designation of 82 CNS tumour classes characterized by distinct DNA methylation profiles (Fig. 1a). Of these, 29 classes were equivalent to a single WHO entity (category 1); 29 classes represented subclasses within a WHO entity (category 2); in 8 classes, WHO grading was not fully recapitulated (category 3) and in 11 classes, the boundaries of methylation classes were not identical to the entity boundaries of WHO (category 4) (Fig. 1a). The remaining five represented DNA methylation classes that have not been defined by the WHO classification (category 5), three of which were recently described[4] as well as the not yet well-defined class of astrocytoma and one new subclass of infantile hemispheric glioma. There was evidence for several additional classes of rare tumours, with too few cases to be included at present. Taking the impact of the tumour microenvironment on the methylation profile into consideration, we included 47 tumour samples with a pronounced inflammatory or reactive tumour microenvironment, both of which have distinct methylation profiles. We additionally selected 72 samples that represent seven non-neoplastic CNS regions, resulting in a combined reference cohort of 2,801 samples from 91 classes (Fig. 1a) that was visualized using *t*-distributed stochastic neighbour embedding (*t*-SNE) dimensionality reduction[17] (Fig. 1b). This analysis further supported the separation of samples into the defined DNA methylation classes (see also Extended Data Fig. 1a, b; unprocessed IDAT files can be downloaded from the NCBI Gene Expression Omnibus (GEO), under accession number GSE109381). Supplementary Table 1 gives an overview of methylation class characteristics and Supplementary Table 2 shows case-by-case information of the reference samples.

A list of authors and affiliations appears in the online version of the paper.

Capper, D. *et al.* DNA methylation-based classification of central nervous system tumours. *Nature* **555**, 469–474 (2018).

Enabling precision tumor diagnostics

- DNA methylation-based classification has become a central pillar of state-of-the-art diagnostics in neuro-oncology.
- Most prominently, the 2021 edition of the World Health Organization classification of central nervous system tumors lists DNA methylation profiling results as a desirable or even **essential criterion** for diagnosing several tumor types.
- Other guideline authorities and medical societies, such as the NCCN, EANO, ICCR or RCPath UK, have also integrated methylation profiling into their recommendations.
- We recently introduced the **Heidelberg CNS Tumor Methylation Classifier version 12.8**, trained using 7,495 methylation profiles, thereby expanding recognized tumor types from 91 classes in the previously published version 11 to **184 subclasses**.
- This expansion is primarily driven by novel tumor types discovered further elucidating the heterogeneity of CNS tumors.



Louis, D. N. *et al.* The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol* **23**, 1231–1251 (2021)
Horbinski, C. et al. NCCN Guidelines® Insights: Central Nervous System Cancers, Version 2.2022. Journal of the National Comprehensive Cancer Network 21, 12–20 (2023)
Sahm, F. et al. Molecular diagnostic tools for the World Health Organization (WHO) 2021 classification of gliomas, glioneuronal and neuronal tumors; an EANO guideline. Neuro Oncol 25, 1731–1749 (2023)
Wesseling P, et al. Tumours of the Central Nervous System (CNS) Reporting Guide. 2nd edition. International Collaboration on Cancer Reporting Preprint at (2024)

Four tier hierarchical structure established

- A four-tier hierarchical structure comprising **superfamilies, families, classes, and subclasses** was introduced.
- Established diagnostic categories generally reside at the family or class level, where sufficient clinical and molecular evidence supports robust demarcation.
- Newly recognized entities - sometimes defined by rather subtle epigenetic variations - are often placed at the subclass level, acknowledging unknown clinical relevance at this early stage.
- In cases where the boundaries among subclasses remain poorly defined, the classifier defaults to higher-level group assignments as a **conservative approach**.
- The hierarchical organization accommodates both well-characterized tumor subtypes and newly discovered clusters, offering a nuanced classification system that captures the current knowledge and complexity of the CNS tumor landscape.

Proposed evidence level annotation established

- The available information on the new and existing classes and subclasses and their alignment with the current WHO classification has been captured in evidence level annotation.
- The annotation has been curated and reviewed among an international group of neuropathologists

    - **Level a - Tumor type/subtype identical to WHO 2021**
    - **Level b - Large single or more than one smaller dataset published describing the type/subtype as molecularly and/or clinically distinct, or the methylation class represents a distinct fraction of an established WHO 2021 tumor class**
    - **Level c - Single small dataset or case series**
    - **Level d - solely based on clusters in tSNE/UMAP**

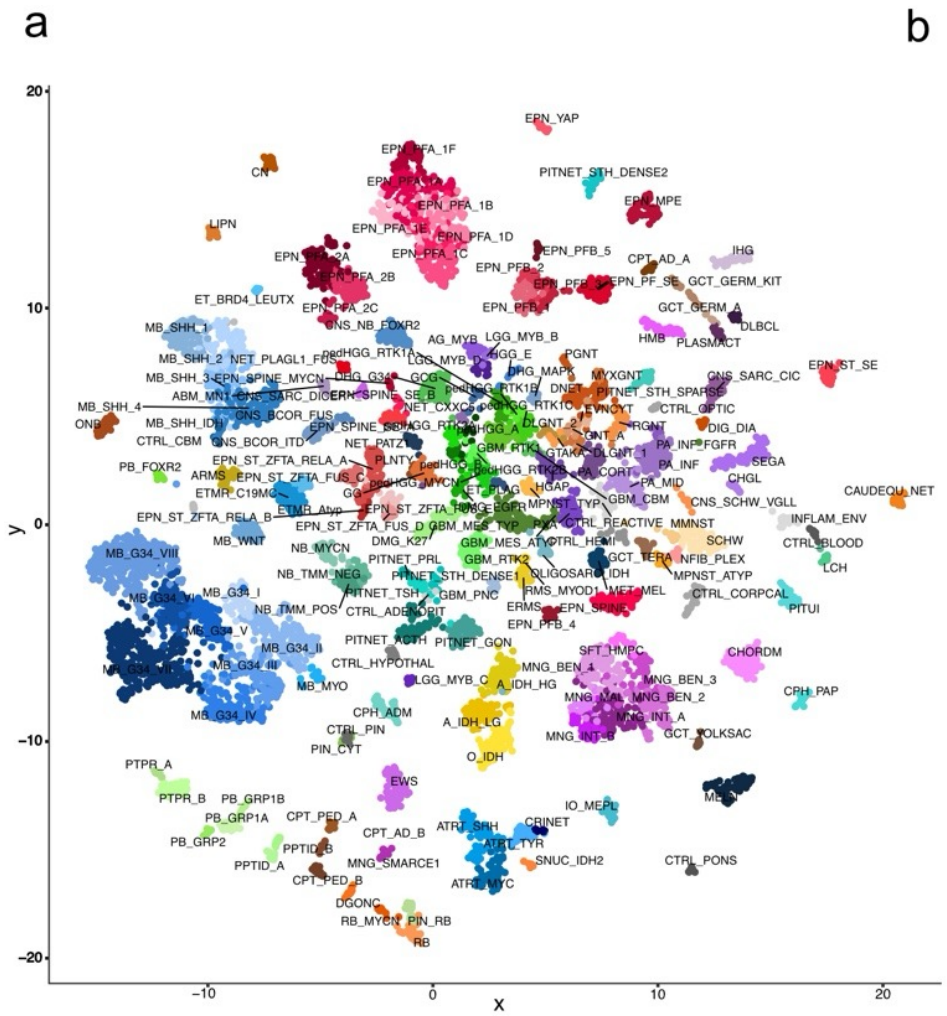
- The annotation is typically provided at the most granular layer, plus in part also a higher layer if that instead matches a WHO type/subtype.

Training and evidence level annotation illustrated

Training dataset version 12.8

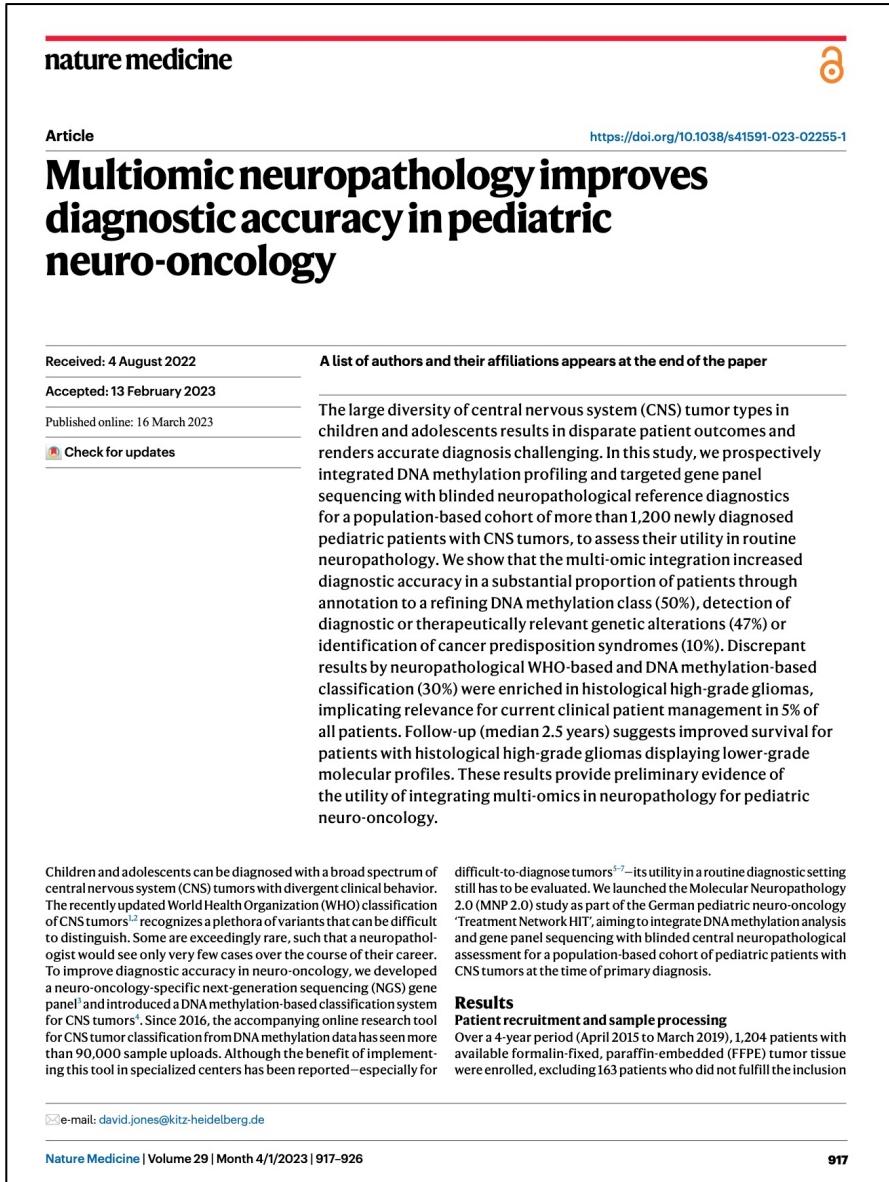
(a) UMAP projection of 7,495 samples used for training.

(b) Legend indicating color code for each subclass. Letters in rounded brackets before abbreviation of the subclass indicate ‘evidence level’.



Independent clinical performance validation

- A prospective study with >1,200 cases showed that adding molecular profiling **improved diagnosis in nearly 50% of cases** compared to the gold standard.
- Classification enabled diagnostics of brain cancer subtypes that are difficult to differentiate by histology
- Classification confirmed treatment selection, including innovative treatments for certain cancer subtypes and therefore supported payer dialogue and fast coverage decisions
- Classification **prevented over-treatment of tumors misclassified as malignant by histology**, reducing treatment costs and patient/family burden



nature medicine

Article

https://doi.org/10.1038/s41591-023-02255-1

Multiomic neuropathology improves diagnostic accuracy in pediatric neuro-oncology

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Check for updates

A list of authors and their affiliations appears at the end of the paper

The large diversity of central nervous system (CNS) tumor types in children and adolescents results in disparate patient outcomes and renders accurate diagnosis challenging. In this study, we prospectively integrated DNA methylation profiling and targeted gene panel sequencing with blinded neuropathological reference diagnostics for a population-based cohort of more than 1,200 newly diagnosed pediatric patients with CNS tumors, to assess their utility in routine neuropathology. We show that the multi-omic integration increased diagnostic accuracy in a substantial proportion of patients through annotation to a refining DNA methylation class (50%), detection of diagnostic or therapeutically relevant genetic alterations (47%) or identification of cancer predisposition syndromes (10%). Discrepant results by neuropathological WHO-based and DNA methylation-based classification (30%) were enriched in histological high-grade gliomas, implicating relevance for current clinical patient management in 5% of all patients. Follow-up (median 2.5 years) suggests improved survival for patients with histological high-grade gliomas displaying lower-grade molecular profiles. These results provide preliminary evidence of the utility of integrating multi-omics in neuropathology for pediatric neuro-oncology.

Children and adolescents can be diagnosed with a broad spectrum of central nervous system (CNS) tumors with divergent clinical behavior. The recently updated World Health Organization (WHO) classification of CNS tumors[1,2] recognizes a plethora of variants that can be difficult to distinguish. Some are exceedingly rare, such that a neuropathologist would see only very few cases over the course of their career. To improve diagnostic accuracy in neuro-oncology, we developed a neuro-oncology-specific next-generation sequencing (NGS) gene panel[3] and introduced a DNA methylation-based classification system for CNS tumors[4]. Since 2016, the accompanying online research tool for CNS tumor classification from DNA methylation data has seen more than 90,000 sample uploads. Although the benefit of implementing this tool in specialized centers has been reported—especially for difficult-to-diagnose tumors[5–7]—its utility in a routine diagnostic setting still has to be evaluated. We launched the Molecular Neuropathology 2.0 (MNP 2.0) study as part of the German pediatric neuro-oncology 'Treatment Network HIT', aiming to integrate DNA methylation analysis and gene panel sequencing with blinded central neuropathological assessment for a population-based cohort of pediatric patients with CNS tumors at the time of primary diagnosis.

Results

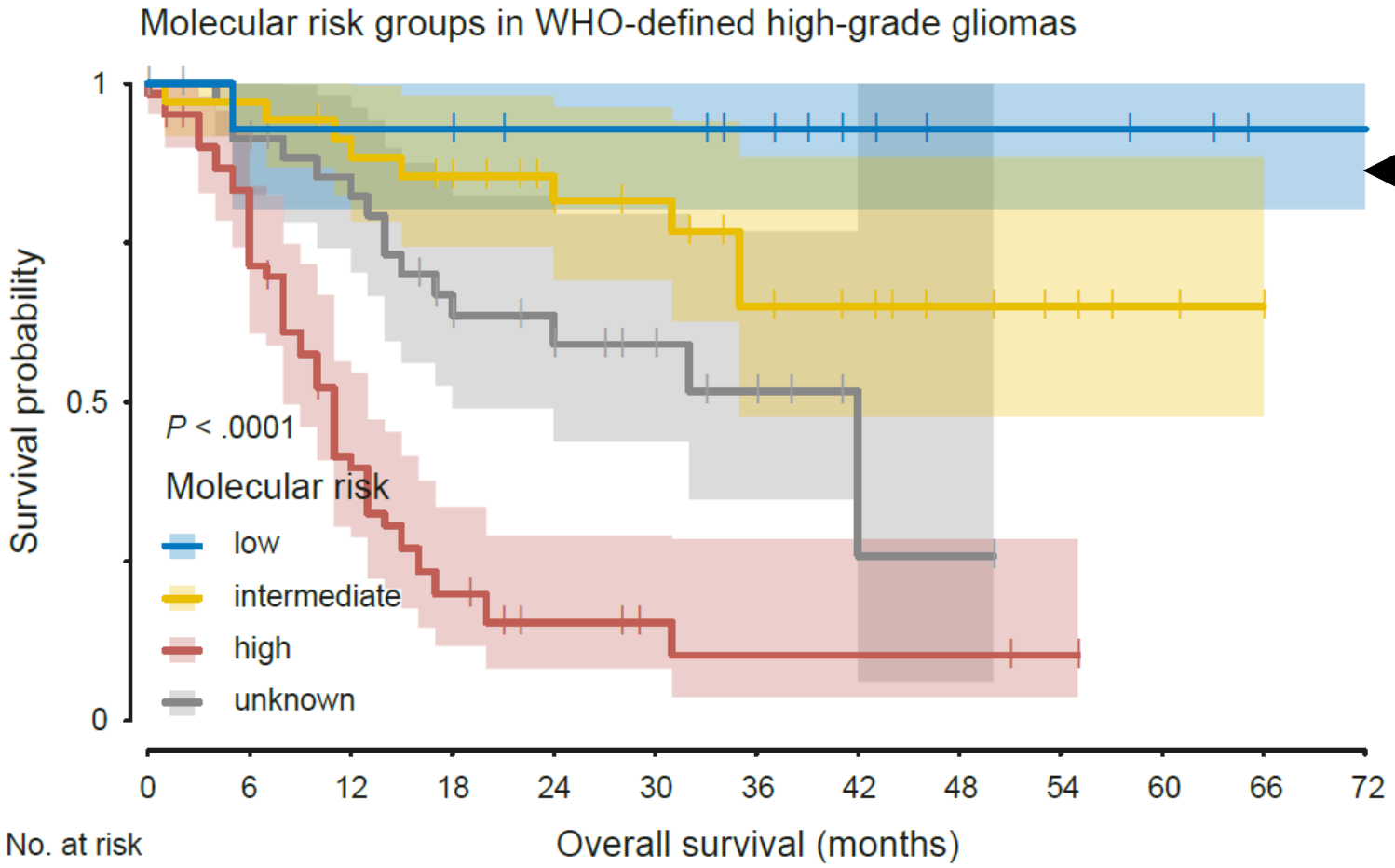
Patient recruitment and sample processing

Over a 4-year period (April 2015 to March 2019), 1,204 patients with available formalin-fixed, paraffin-embedded (FFPE) tumor tissue were enrolled, excluding 163 patients who did not fulfill the inclusion

e-mail: david.jones@kitz-heidelberg.de

Sturm, D. *et al.*; *Nat Med* **29**, 917–926 (2023).

Improved treatment outcome



tumors originally diagnosed as ‘high-grade glioma’ according to standard practices

But they are molecular low-grade with excellent survival → **unnecessary over-treatment** with chemo- and radiotherapy could be avoided

Example of an **independent validation** of classifier utility

Sturm, D. *et al. Nat Med* **29**, 917–926 (2023).

Novel entities defined: DGONC

Neuropathology and Applied Neurobiology (2020), **46**, 422–430 doi: 10.1111/nan.12590

Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters (DGONC) – a molecularly defined glioneuronal CNS tumour class displaying recurrent monosomy 14

M. Y. Deng[1,2], M. Sill[1,3], D. Sturm[1,2,4], D. Stichel[5,6], H. Witt[1,3,4], J. Ecker[1,4,7], A. Wittmann[1,2], J. Schittenhelm[8], M. Ebinger[9], M. U. Schuhmann[10], D. Figarella-Branger[11], E. Aronica[12], O. Staszewski[13], M. Preusser[14], C. Haberler[15], M. Lauten[16], U. Schüller[17], C. Hartmann[18], M. Sn C. Dunham[20], N. Jabado[21,22], P. Wesseling[23,24], M. Deckert[25], K. Keyvani[26], N. Gottardo[27,28,2 Giangaspero[30,31], K. von Hoff[32], D. W. Ellison[33], T. Pietsch[34], C. Herold-Mende[35], T. Milde[1,4,] Witt[1,4,7], M. Kool[1,3], A. Korshunov[5,6], W. Wick[36,37], A. von Deimling[5,6], S. M. Pfister[1,3,4], D. T. Jones[1,2,1] and F. Sahm[1,5,6]*

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Aims: DNA methylation-based central nervous system (CNS) tumour classification has identified numerous molecularly distinct tumour types, and clinically relevant subgroups among known CNS tumour entities that were previously thought to represent homogeneous diseases. Our study aimed at characterizing a novel, molecularly defined variant of glioneuronal CNS tumour. **Patients and**

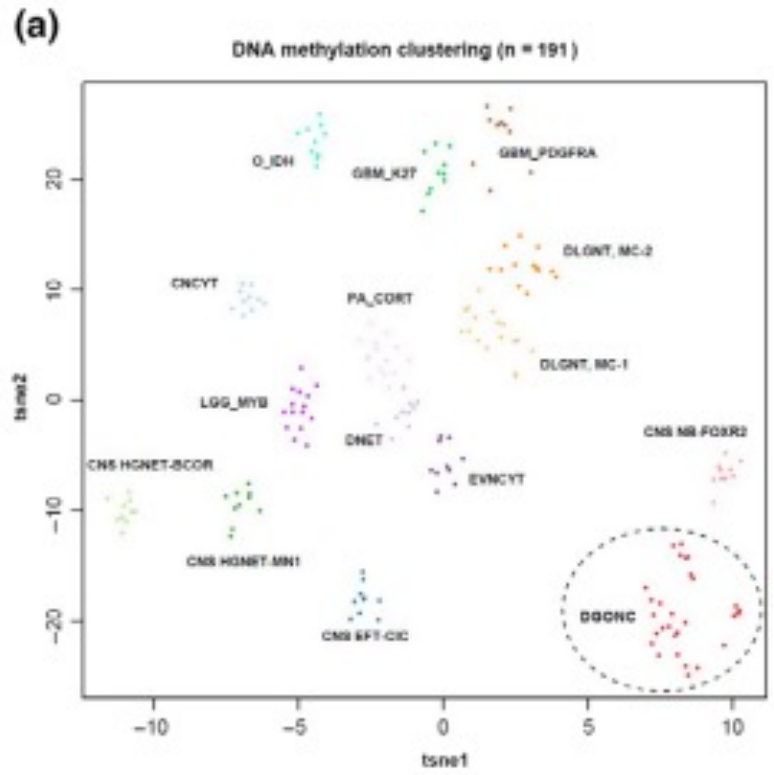
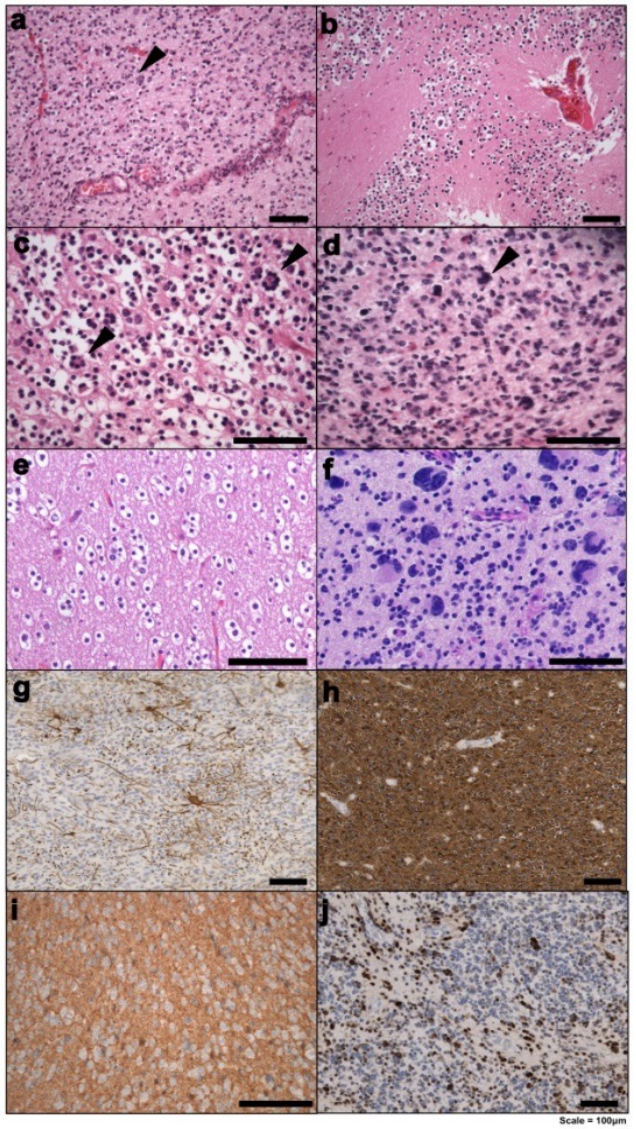


Figure 1. DNA methylation profiling of DGONC.

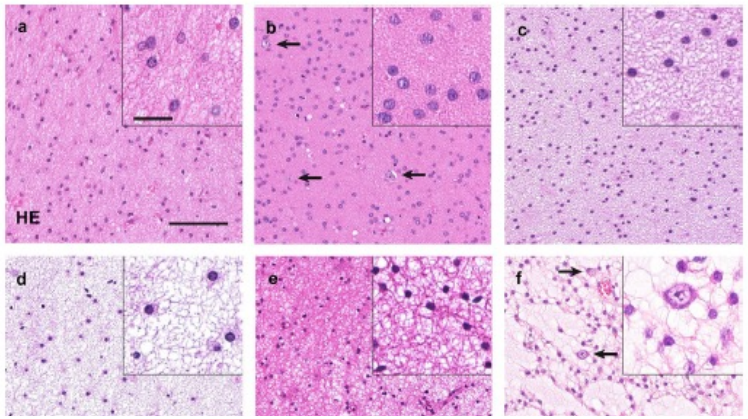


Deng et al., NAN 2020

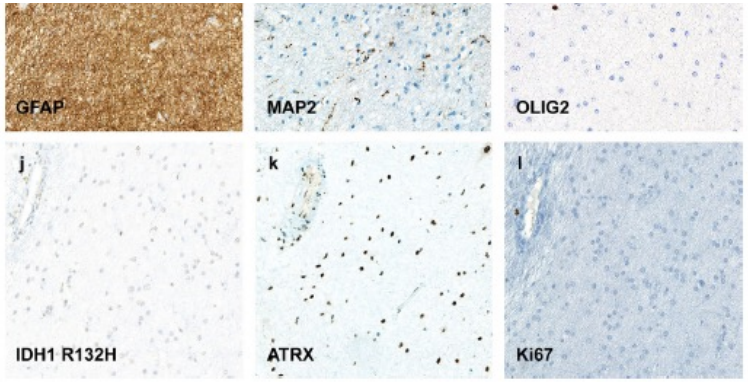
Novel entities confirmed: IDG

Acta Neuropathol. 2020 January ; 139(1): 193–209. doi:10.1007/s00401-019-02078-w.

Isomorphic diffuse glioma is a morphologically and molecularly distinct tumour entity with recurrent gene fusions of *MYBL1* or *MYB* and a benign disease course



In this study, we demonstrate that isomorphic diffuse glioma is an IDH-wildtype tumour class that is molecularly distinct from other established glial/glio-neuronal tumour entities, belongs to the family of *MYB*/*MYBL1*-altered gliomas and should be included in the differential diagnoses of low-grade epilepsy-associated tumours in both children and adults.



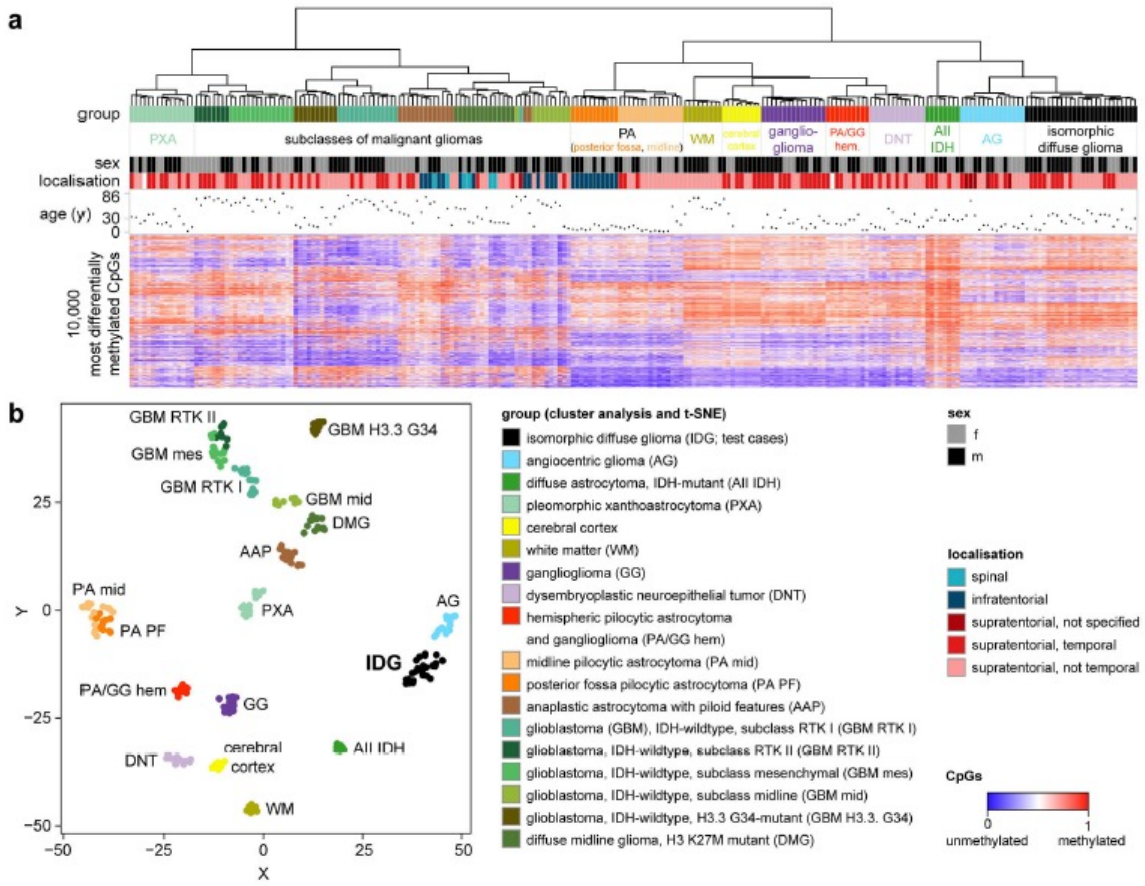


Fig. 3.
Isomorphic diffuse glioma forms a distinct methylation cluster. a Unsupervised hierarchical

Wefers et al., ANP 2020

Example Report Methylation Profiling, MGMT-PM and CNV

Methylation Profiling Report

This report has been generated using the **CNS Tumor Methylation Classifier v12.8**. Detailed information about the classifier and description of classes is available at https://app.epignostix.com.

About the **CNS Tumor Methylation Classifier v12.8**:

The classifier was trained based on 7,495 methylation profiles, comprising 184 tumor classes. Utilizing a Random Forest–based methodology, the classifier was rigorously validated through five-fold nested cross-validation, achieving a 95% subclass-level accuracy and a Brier Score of 0.028, indicative of well-calibrated probability estimates. The hierarchical output structure facilitates comprehensive interpretation, allowing clinicians to assess subclass and aggregate class-level probabilities for informed diagnostic decisions. Comparative analyses demonstrate that v12.8 surpasses previous versions and traditional WHO-based diagnostics in prognostic performance across diverse tumor cohorts. Established and distributed under the Molecular Neuropathology (MNP) banner, the CNS Tumor Methylation Classifier has been widely used since 2017.

Sample Information

Sample Identifier	Sentrix ID	Array type	Material type	Biological Sex
[redacted]		epic	Frozen	Male

Methylation Classifier

Level	Prediction	Calibrated Score	Interpretation		Evidence
Superfamily	Ependymal Tumours	0.9999	match	✓	
Family	Spinal Ependymoma	0.9999	match	✓	
Class	Spinal Ependymoma, MYCN-Amplified	0.9999	match	✓	
Subclass	Spinal Ependymoma, MYCN-Amplified	0.9999	match	✓	A

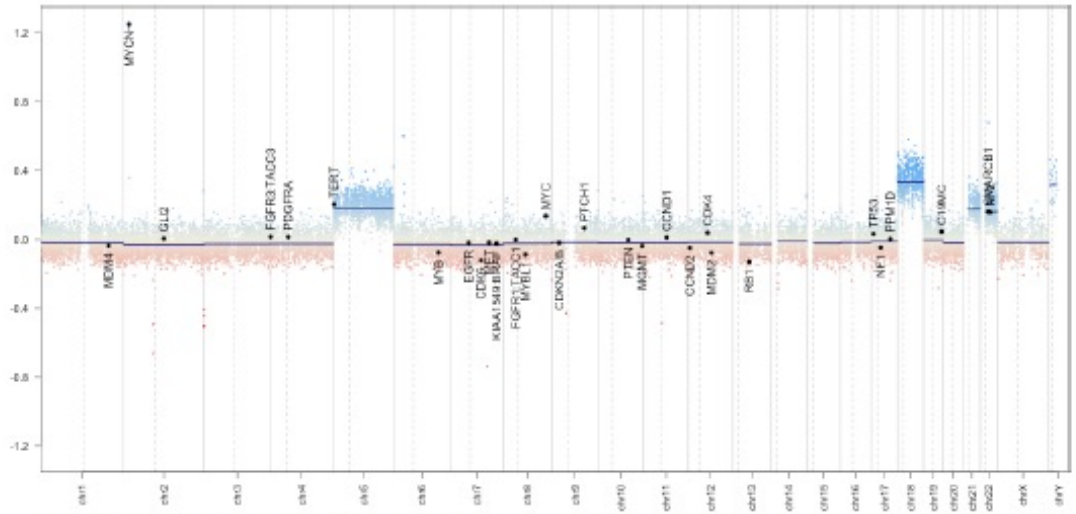
Interpretation Symbol:
✓ match (score $\geq$ 0.9)
✗ no match (score $<$ 0.9)

Evidence Level WHO:
Level A Tumor type/subtype identical to WHO 2021.
Level B Large single or more than one smaller dataset published describing the type/subtype as molecularly and/or clinically distinct, or the methylation class represents a distinct fraction of an established WHO 2021 tumor class.
Level C Single small dataset or case series.
Level D Solely based on clusters in tSNE/UMAP.

Description

The "mc spinal ependymoma, MYCN-amplified" mainly includes tumors with the histological diagnosis of anaplastic ependymoma. Average age is younger than that of "mc spinal ependymoma. Most cases carry MYCN amplification but no NF2 mutation. The clinical course of these tumours appears aggressive.

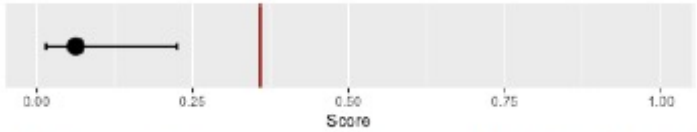
Copy Number Variation Profile



Generation of the copy number profile of the sample is based on conumee2 (Daenekas et. al 2024). The profile depicts log2 transformed copy number ratios (Y-axis) for chromosomes 1 through 22 (and X/Y if automatic prediction was successful). Potential gains or amplifications are represented by the positive Y-axis, while losses are depicted by the negative Y-axis. 29 CNS tumor relevant gene regions are highlighted.

Bjarne Daenekas, Eilís Pérez, Fabio Boniolo, Sabina Stefan, Salvatore Benfatto, Martin Sill, Dominik Sturm, David T W Jones, David Capper, Marc Zapatka, Volker Hovestadt *Conumee 2.0: enhanced copy-number variation analysis from DNA methylation arrays for humans and mice* Bioinformatics, Volume 40, Issue 2, February 2024.

MGMT Promoter Methylation (MGMT-STP27)



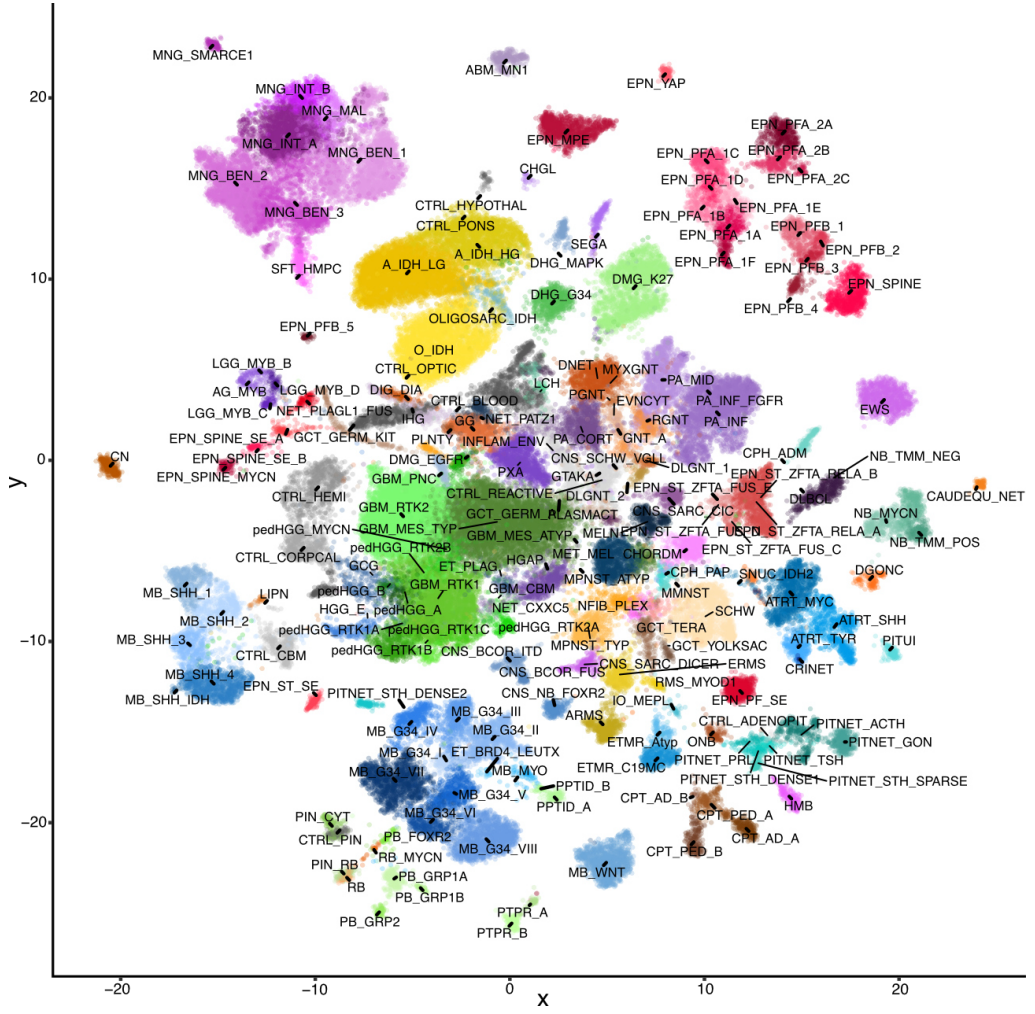
MGMT promoter methylation status is based on the model developed by Bady et. al 2016. Score of the sample is depicted by the black dot and confidence intervals are shown as whiskers. Red line indicates cutoff for assigning methylation status.

Pierre Bady, Davide Sciuscio, Annie-Claire Diserens et al. *MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status.* Acta Neuropathologica, p.547-560, Number 4, 2012.

Broad application across all cancer types

- The biological principles underlying methylation- based tumor classification have the potential to **transform diagnostic practice for ALL types of human cancer**

- Preliminary data on classification for other tumor indications, including head&neck, breast, colon, lung, skin, prostate and CUP has been generated



Revolutionizing Cancer Diagnostics

HEIDELBERG EPIGNOSTIX

Enabling Precision Oncology

Diagnostic AI-powered models assign patient samples precisely and objectively to cancer classes to guide optimal patient treatment

Technology Deployed in Routine Testing

More than **180,000 patient samples** analyzed to date demonstrate rapid adoption and a high unmet clinical need

Thank you

DKFZ, Uniklinik Heidelberg
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Heidelberg Epignostix
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