Applications of Artificial Intelligence in Brain Tumor Diagnosis

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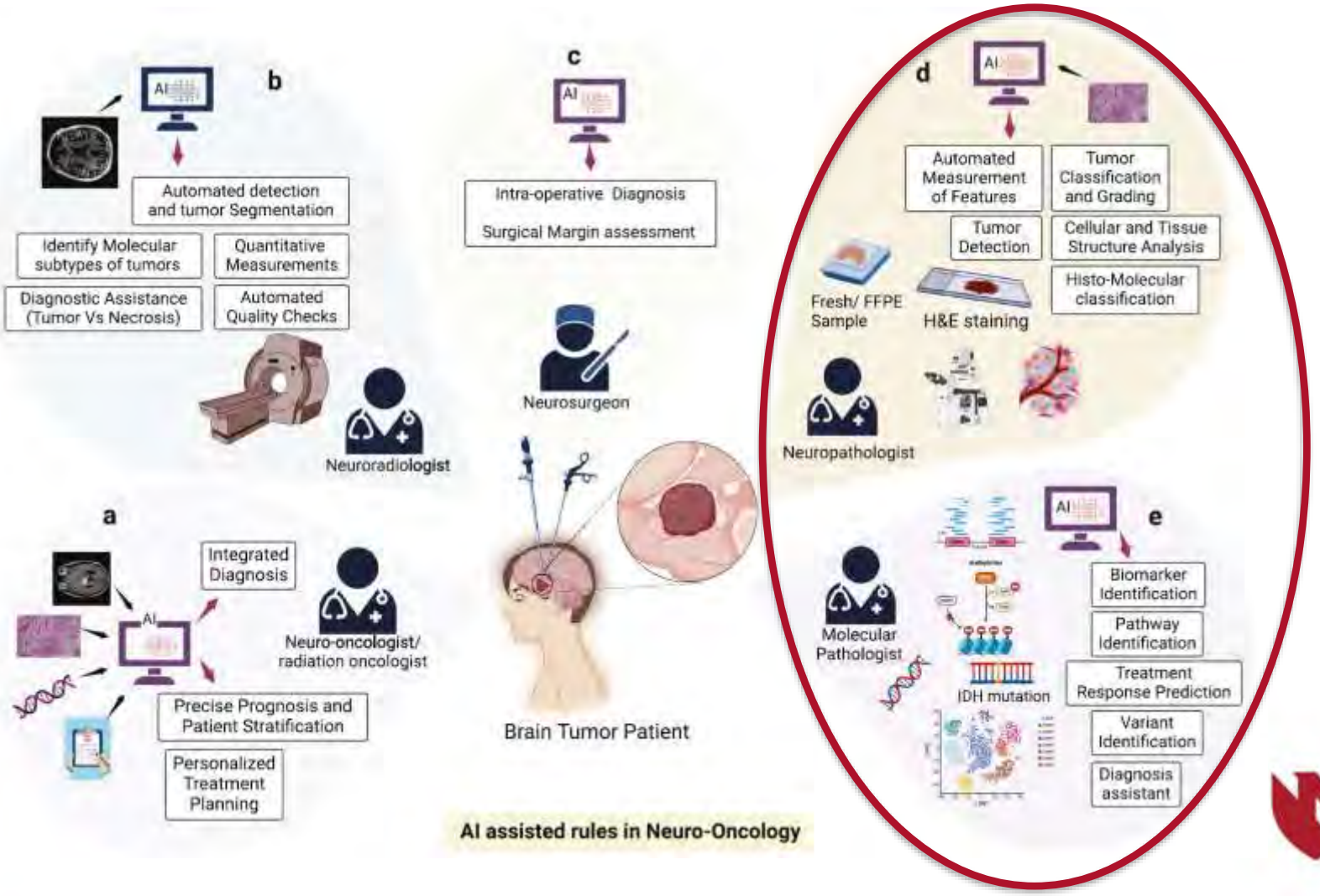
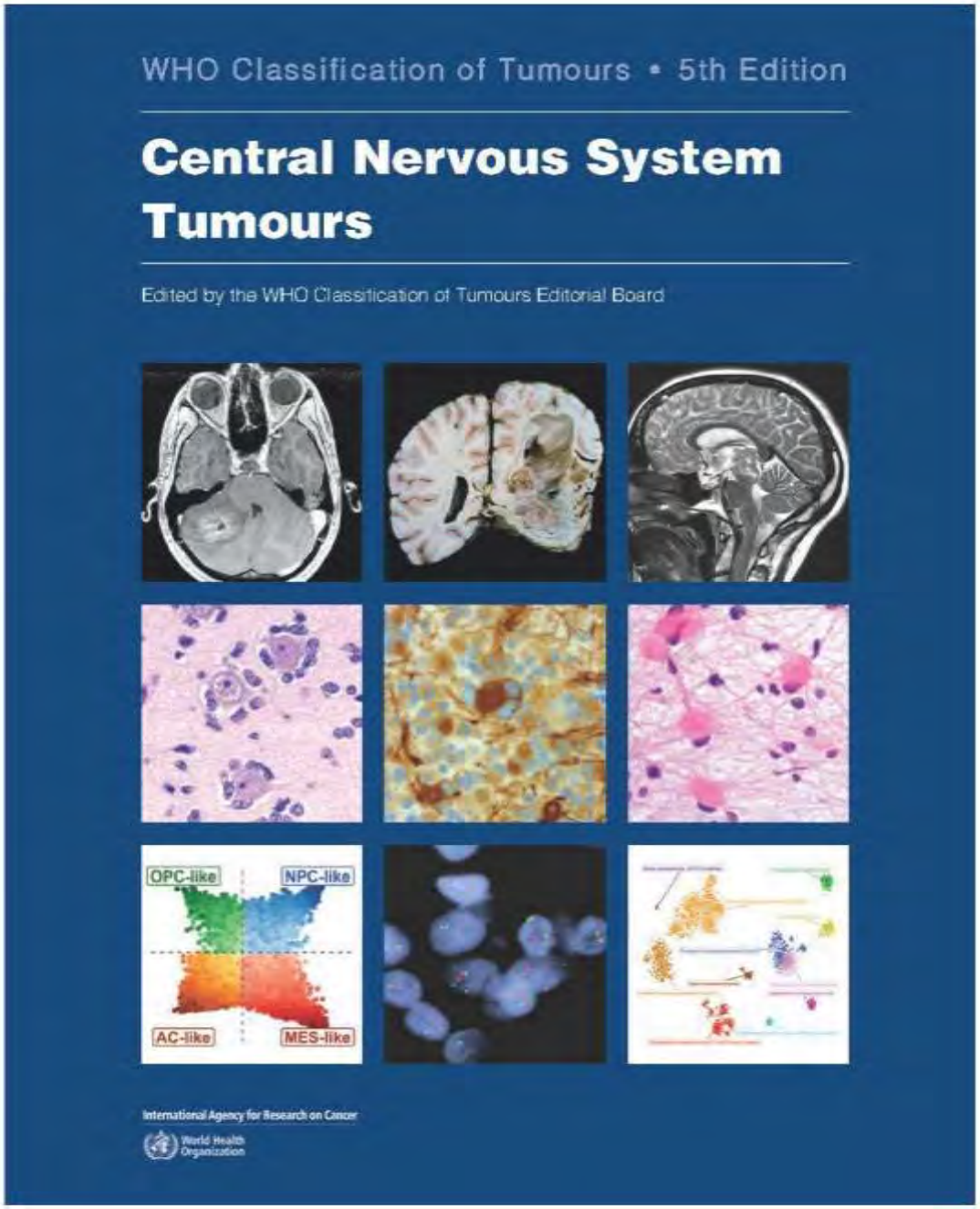
I have no conflicts of interest to disclose.

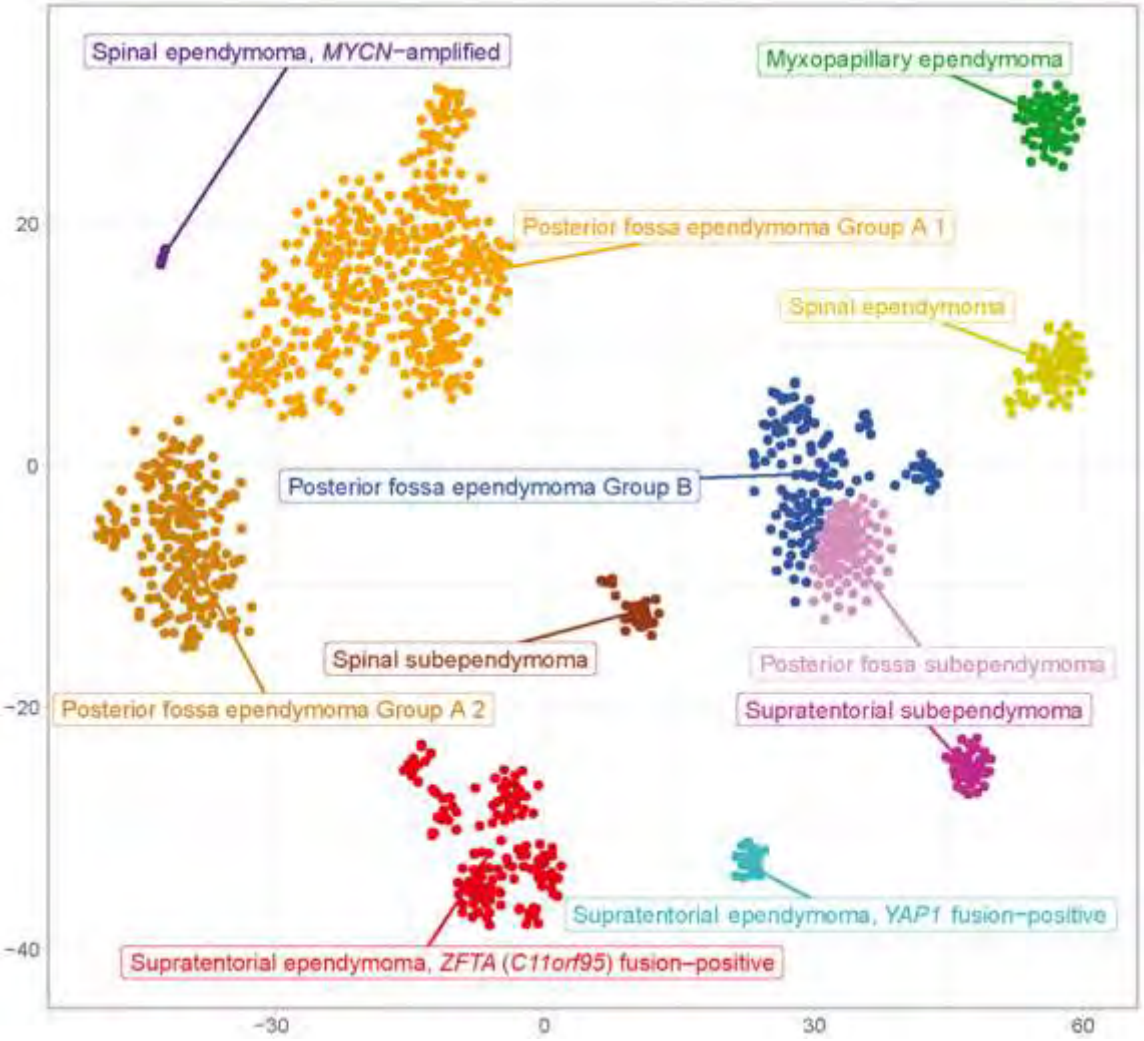
AI in Neuro-Oncology



Khalighi S, Reddy K, Midya A, Pandav KB, Madabhushi A, Abedalthagafi M. Artificial intelligence in neuro-oncology: advances and challenges in brain tumor diagnosis, prognosis, and precision treatment. NPJ Precis Oncol. 2024;8(1):80.


WHO Classification of Tumours • 5th Edition
Central Nervous System Tumours
Edited by the WHO Classification of Tumours Editorial Board
OPC-like
NPC-like
AC-like
MES-like
International Agency for Research on Cancer
World Health Organization

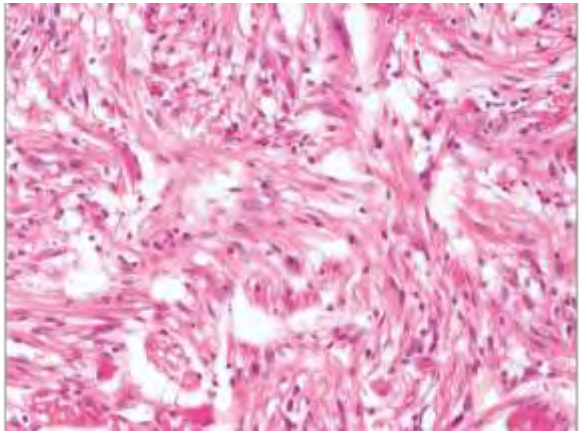
t-SNE projection of methylation array profiles from ependymal tumours

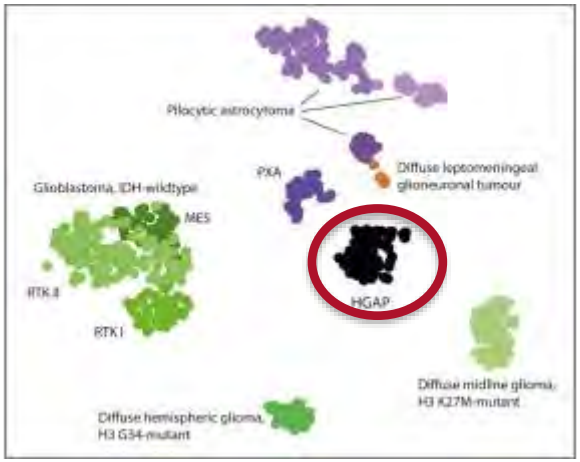


T-SNE: t-distributed stochastic neighbor embedding

2021 WHO

High-grade astrocytoma with piloid features (HGAP)





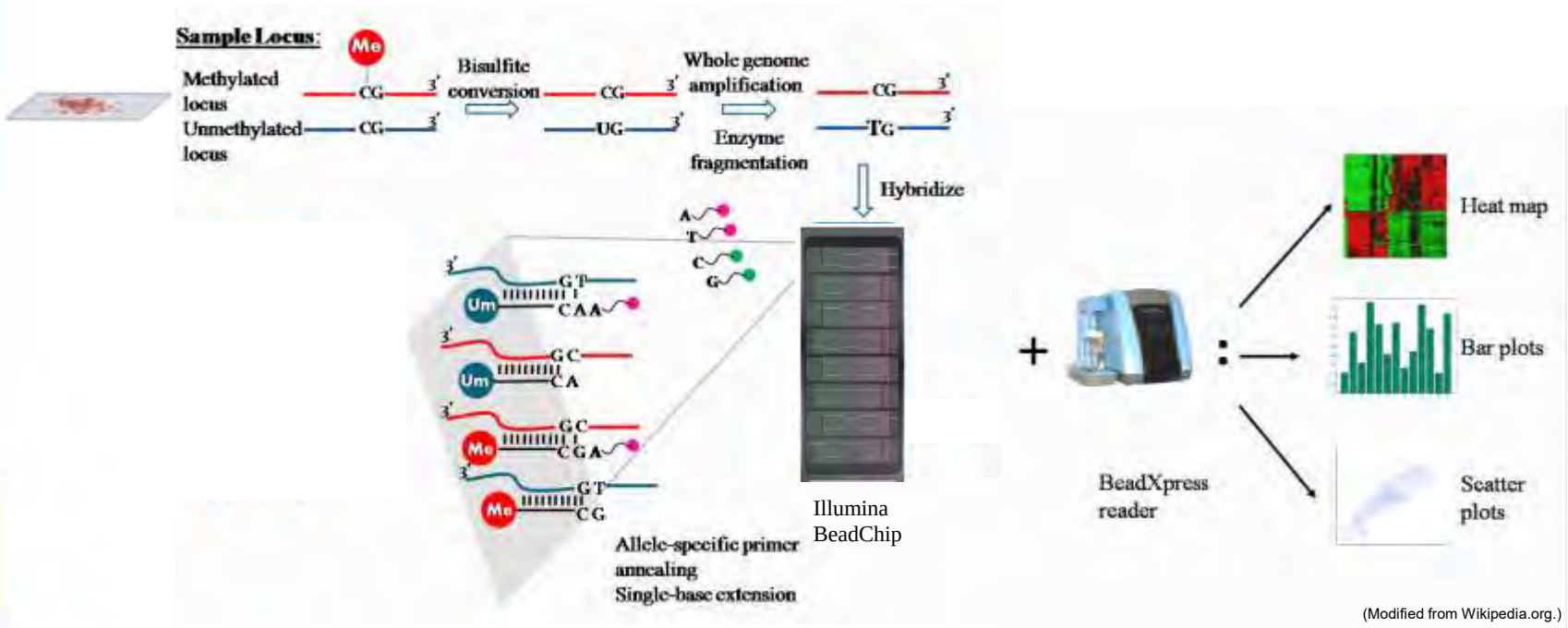
#20524

Diagnostic criteria for high-grade astrocytoma with piloid features

Essential:
An astrocytic glioma
AND
A DNA methylation profile of high-grade astrocytoma with piloid features
Desirable:
MAPK pathway gene alteration
Homozygous deletion or mutation of *CDKN2A* and/or *CDKN2B*, or amplification of *CDK4*
Mutation of *ATRX* or loss of nuclear ATRX expression
Anaplastic histological features

2021 WHO

Infinium MethylationEPIC (850K) BeadChip microarray



1. 850,000 CpG (cytosin-phosphate-guanine) sites
2. Bisulfite conversion:
   - Any methylated C stays as a C
   - Any unmethylated C is converted to a U (uracil)
3. Hybridization on BeadChip, fluorescently labeled and scanned
4. Analysis through bioinformatic pipelines

DNA methylation-based classification of CNS tumors (DKFZ Heidelberg)

ARTICLE

doi:10.1038/nature26000

DNA methylation–based classification of central nervous system tumours

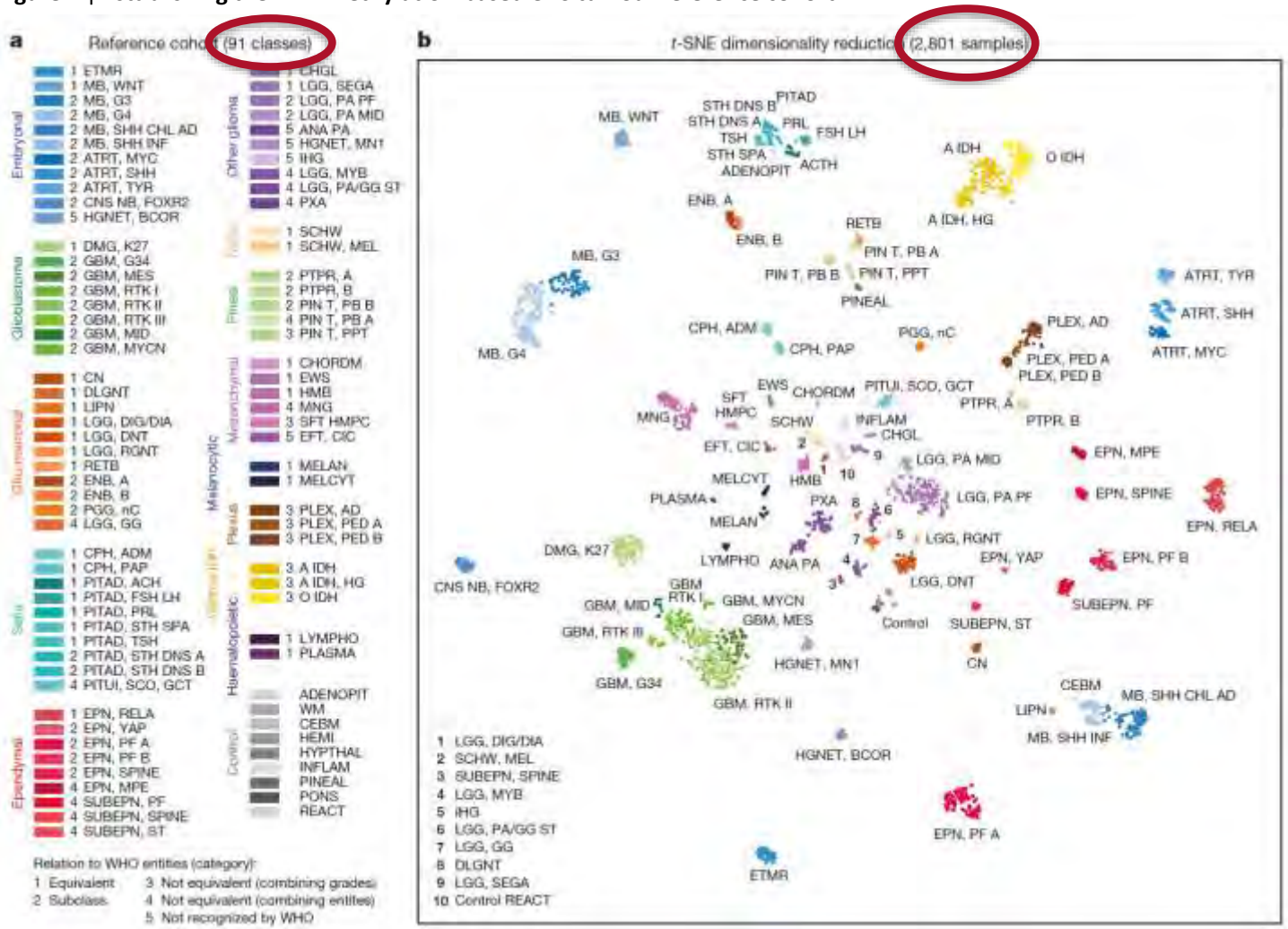
A list of authors and their affiliations appears in the online version of the paper.

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.

Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469-74.

CNS tumor reference cohort

Figure 1 | Establishing the DNA methylation-based CNS tumour reference cohort.



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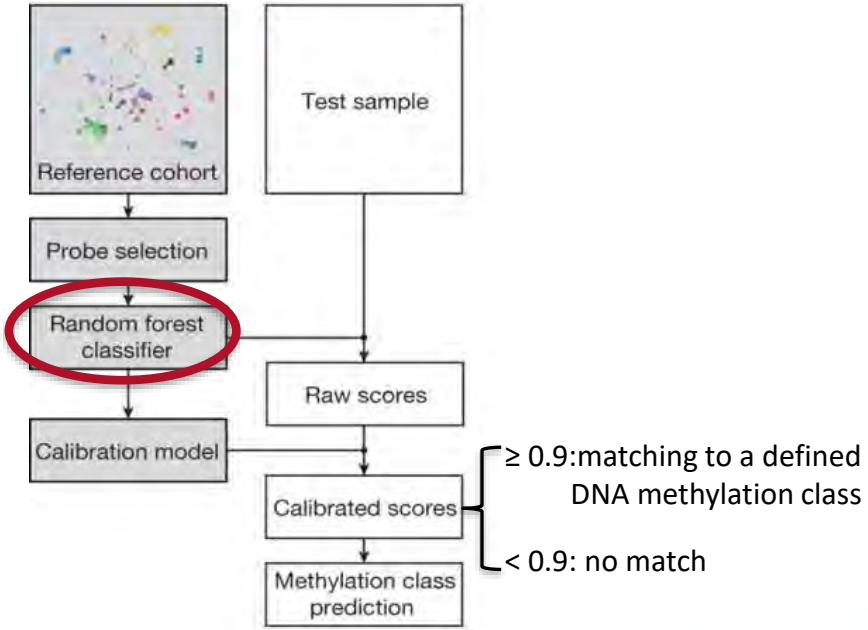
DNA methylation-based classification of CNS tumors (DKFZ Heidelberg)

Figure 2 | Development and cross-validation of the DNA methylation-based CNS tumour classifier.

1. Machine learning random forest classifier
2. Generated 10,000 binary decision trees with data from all 2801 references samples
3. Each tree assigns a given tumor sample to one of the 91 classes resulting in an aggregate raw score
4. Raw scores are transformed calibrated scores to enable inter-class comparability
5. Calibrated scores represent an estimated probability measure of methylation class assignment.



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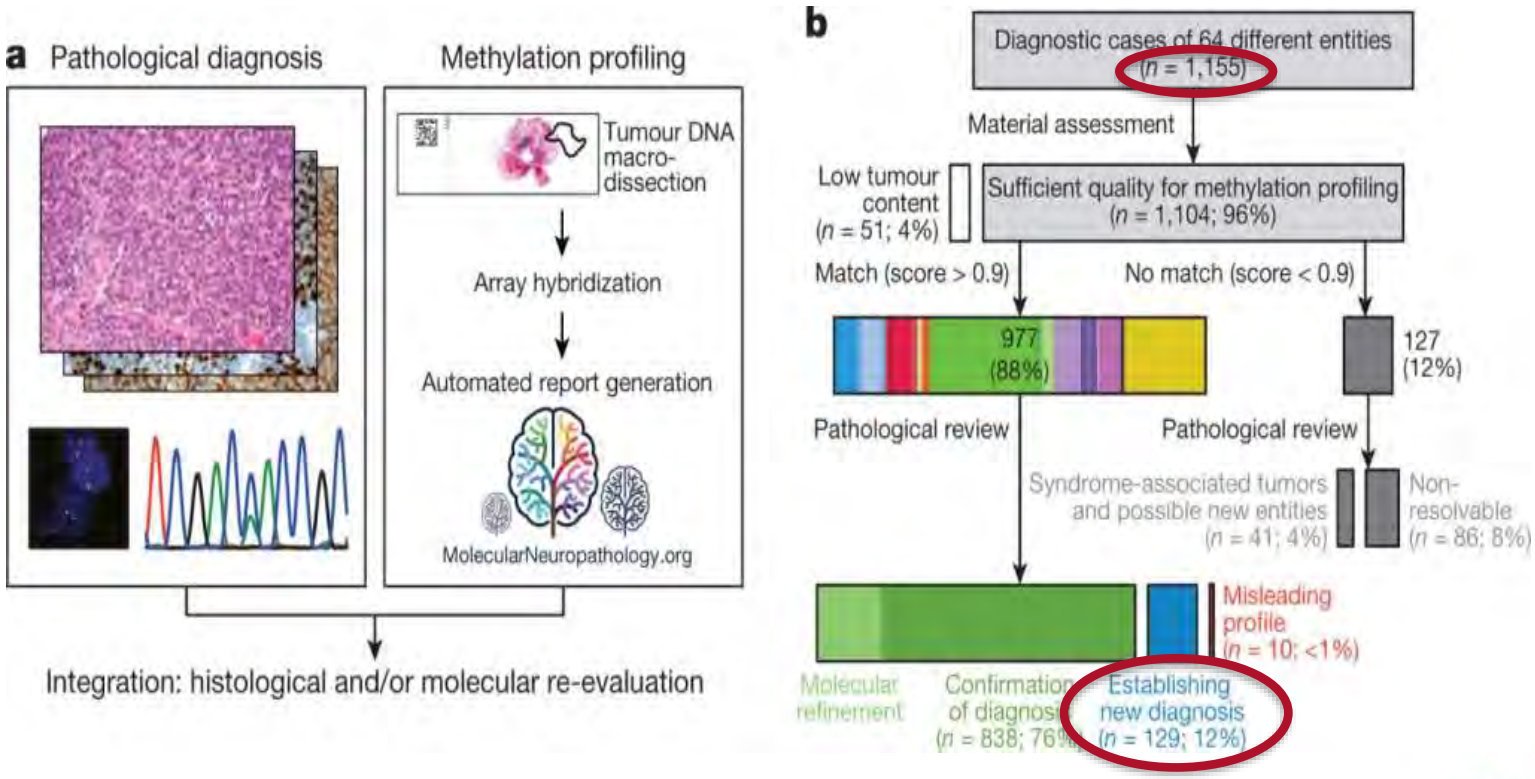
DNA methylation-based classification of CNS tumors (DKFZ Heidelberg)

Figure 3 | Implementation of the classifier in diagnostic practice.



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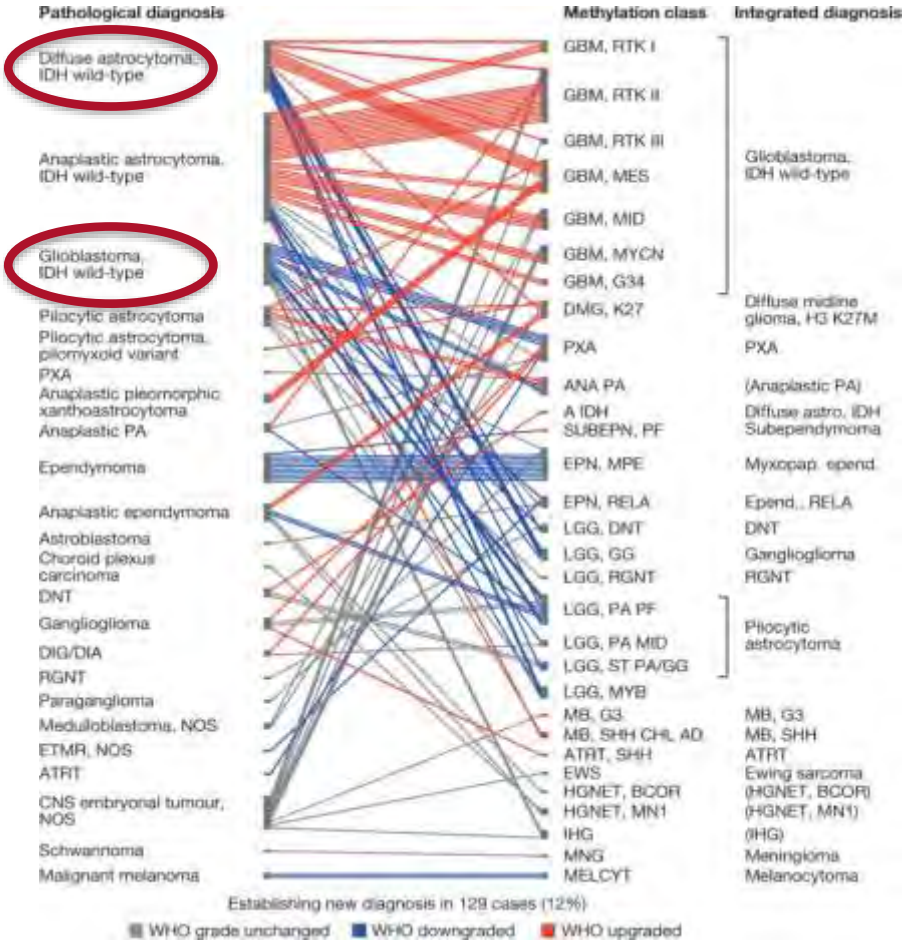
DNA methylation-based classification of CNS tumors (DKFZ Heidelberg)

Figure 4. Reassessment of discrepant cases and establishment of new diagnosis



Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469-74.

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DNA methylation-based classification of CNS tumors (NIH/Bethesda)

Neuro-Oncology

Impact of the methylation classifier and ancillary methods on CNS tumor diagnostics

Zhichao Wu, Zied Abdullaev, Drew Pratt, Hye-Jung Chung, Shannon Skarshaug, Valerie Zgonc, Candice Perry, Svetlana Peck, Lola Saidkhodjaeva, Sushma Nagaraj, Manoj Tyagi, Vineela Gangalapudi, Kristin Valdez, Rust Turakulov, Liqiang Xi, Mark Raffeld, Antonios Papanicolau-Sengos, Kayla O'Donnell, Michael Newford, Mark R. Gilbert, Felix Sahm, Abigail K. Suwala, Andreas von Deimling, Yasin Mamatjan, Shirin Karimi, Farshad Nassiri, Gelareh Zadeh, Eytan Ruppin, Martha Quezado, and Kenneth Aldape

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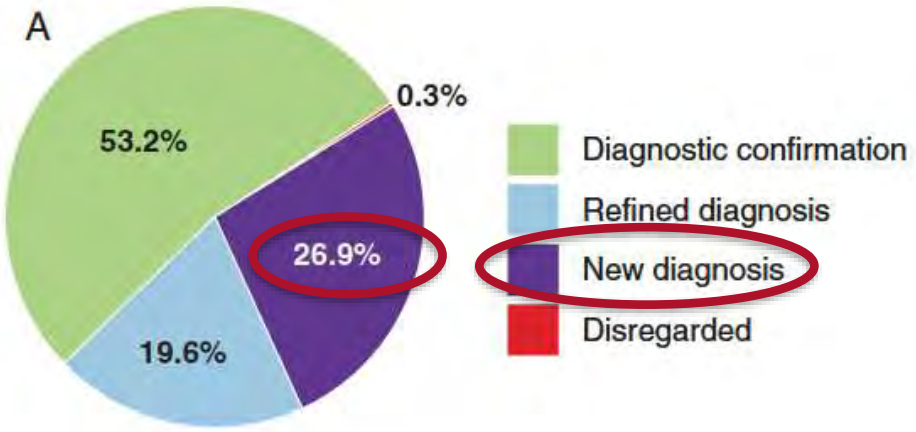
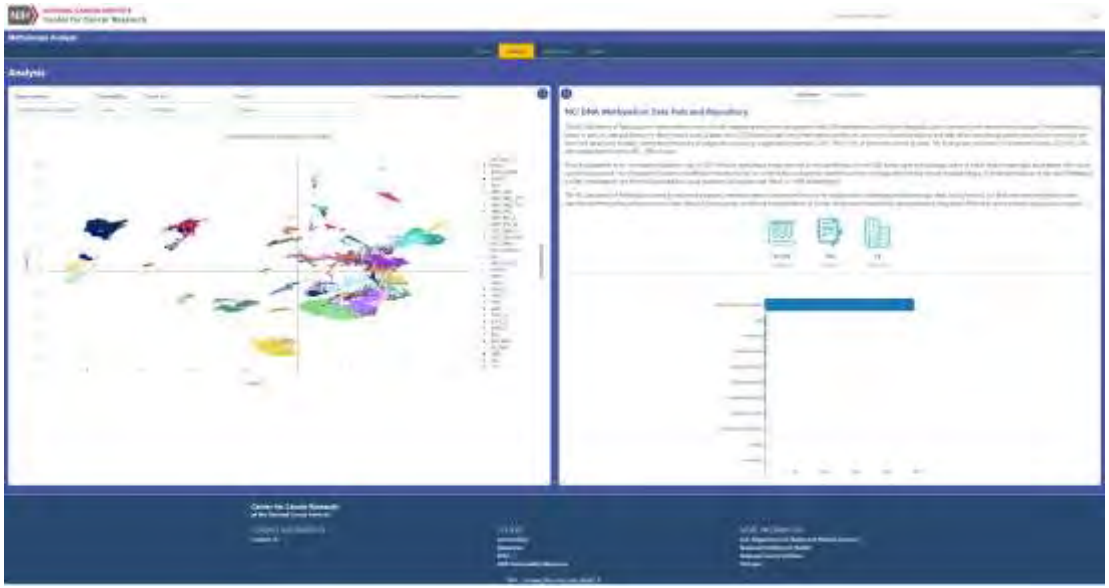
These authors contributed equally to this work.

Among the received cases in consultation, a high-confidence methylation classifier score (>0.84) was reached in 66.4% of cases. The classifier impacted the diagnosis in 46.7% of these high-confidence classifier score cases, including a substantially new diagnosis in 26.9% cases.

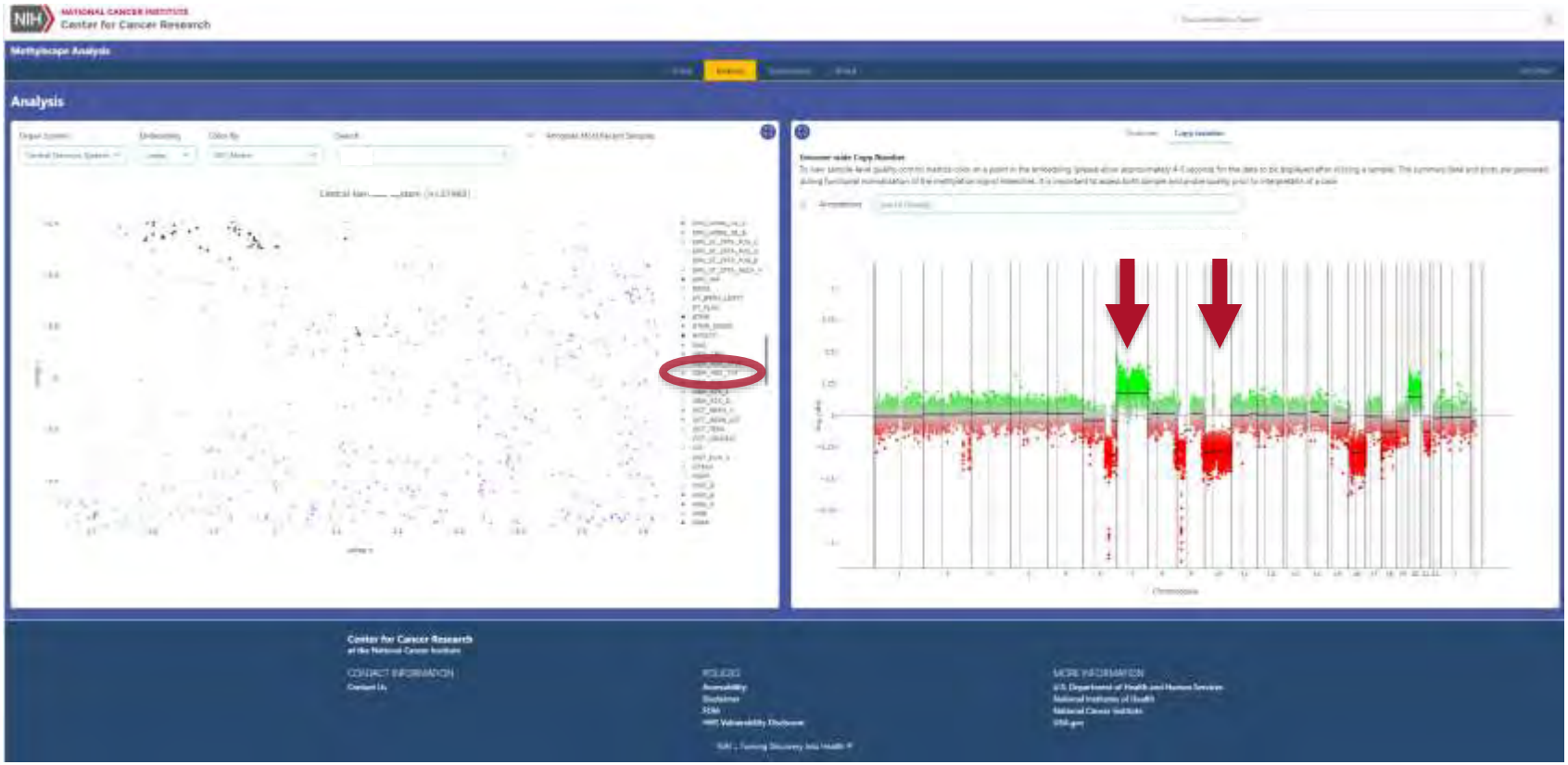
Among the 289 cases received with only a descriptive diagnosis, methylation was able to resolve approximately half (144, 49.8%) with high-confidence scores. Additional methods were able to resolve diagnostic uncertainty in 41.6% of the low-score cases.



MethylScape Analysis-NIH



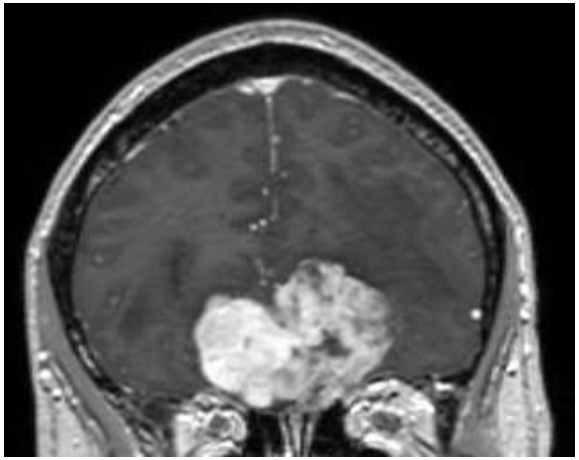
MethylScape Analysis-NIH



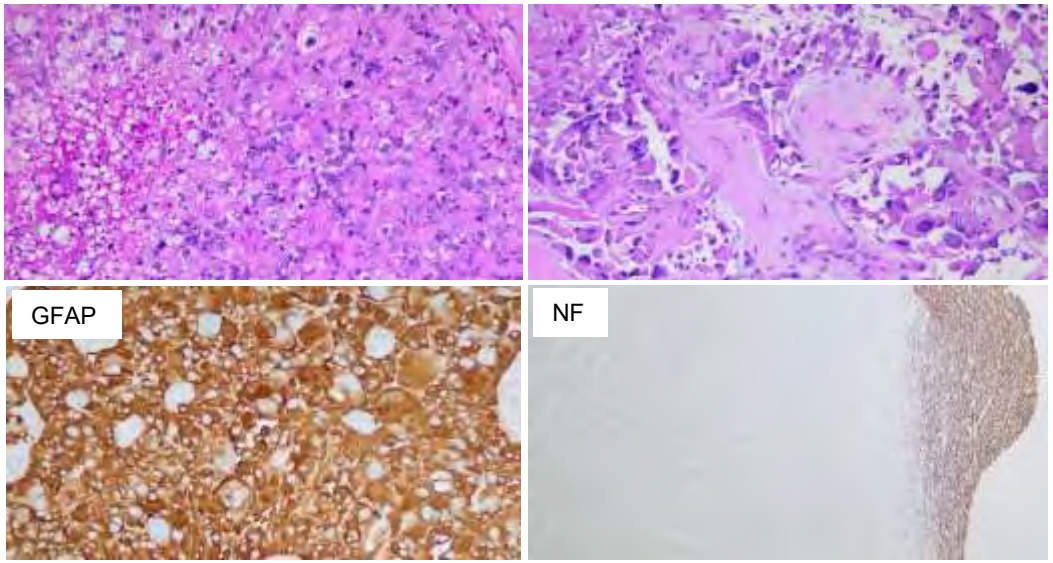
Benefits of methylation profiling

1. Accurately classify brain tumors into specific molecular tumor types
2. Identify new tumor types

UNMC Case presentation



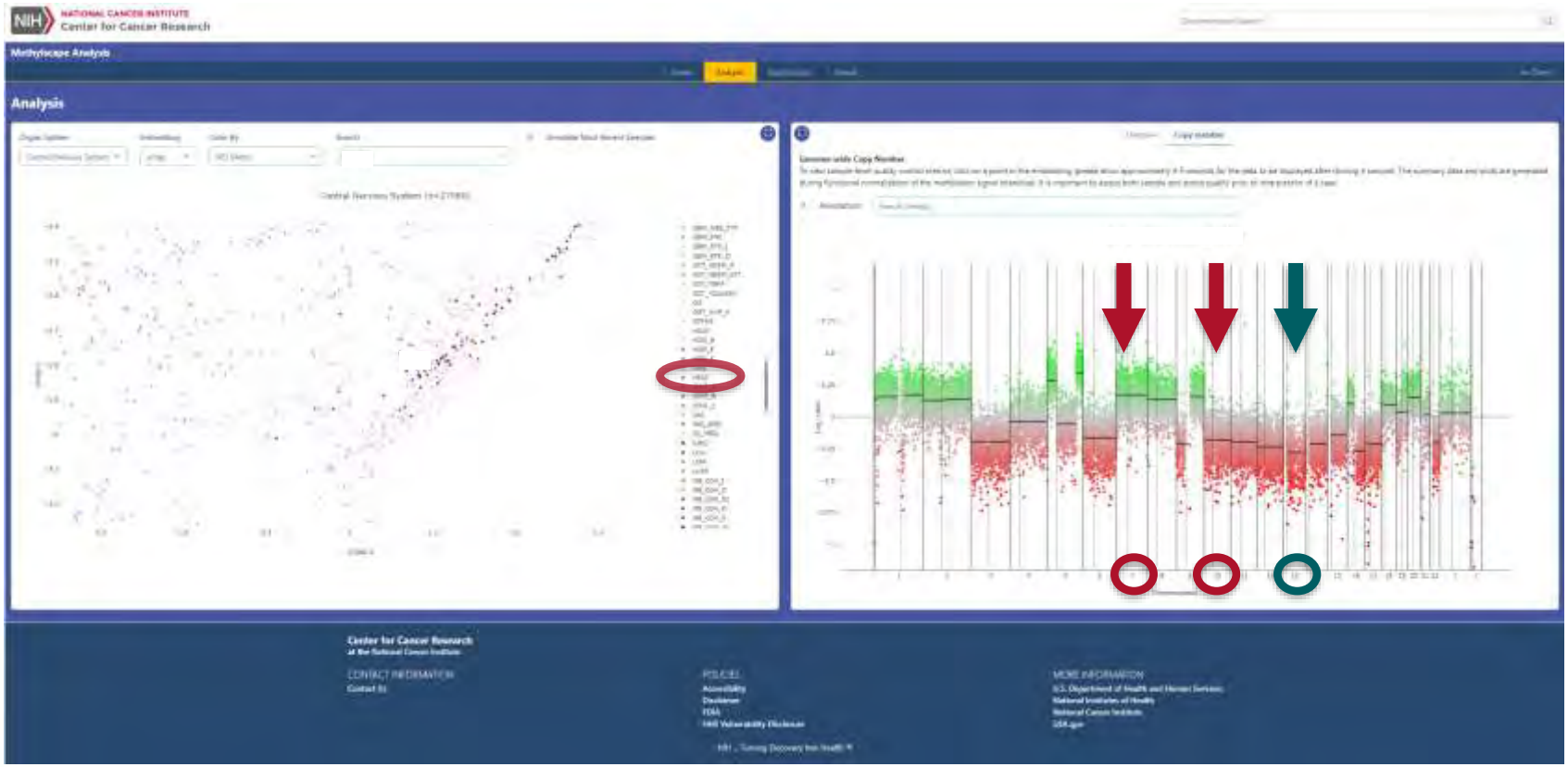
63-year-old male with a 5.8 cm heterogeneously enhancing mass along the anterior inferior frontal lobe



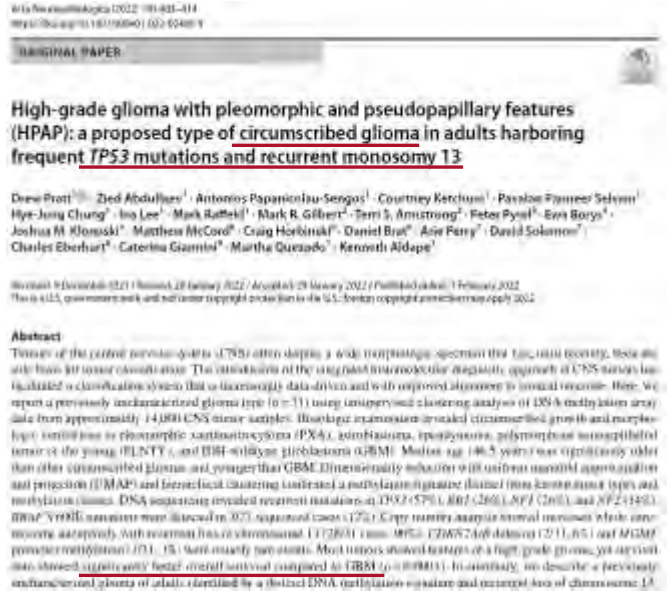
FISH: polysomy 7, monosomy 10, homozygous deletion of CDKN2A

NGS (POP300): *RB1* and *TP53* mutations

MethylScape Analysis-NIH



High-grade glioma with pleomorphic and pseudopapillary features (HPAP)



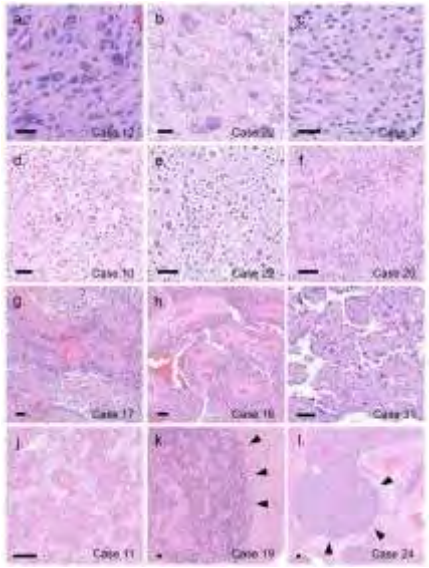
ORIGINAL PAPER

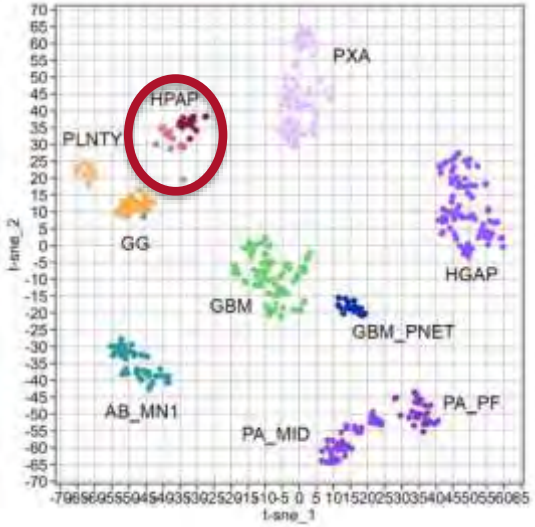
High-grade glioma with pleomorphic and pseudopapillary features (HPAP): a proposed type of circumscribed glioma in adults harboring frequent *TP53* mutations and recurrent monosomy 13

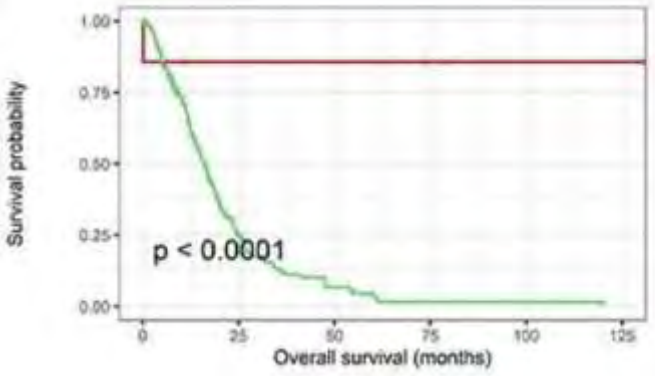
Drew Pratt · Zied Abdullaev · Antonios Papanicolau-Sengos · Courtney Ketchum · Pavalee Panneer Selvam · Hye-Jung Chung · Ina Lee · Mark Raffeld · Mark R. Gilbert · Terri S. Armstrong · Peter Pytel · Eva Borys · Joshua M. Klonoski · Matthew McCord · Craig Horbinski · Daniel Brat · Ace Perry · David Solomon · Charles Eberhart · Caterina Giannini · Martha Quezado · Kenneth Aldape

Abstract

Tumors of the central nervous system (CNS) often display a wide morphologic spectrum that can cause diversity, even the most experienced neuropathologists. The introduction of the integrated histomolecular diagnostic approach in CNS tumors has facilitated a classification system that is increasingly data-driven and with improved alignment to clinical outcome. Here, we report a previously unrecognized glioma type (n = 31) using unsupervised clustering analyses of DNA methylation array data from approximately 14,000 CNS tumor samples. Histologic examination revealed circumscribed growth and morphologic similarities to pleomorphic xanthoastrocytoma (PXA), astroblastoma, ependymoma, polymorphous neuroepithelial tumor of the young (PLNTY), and IDH-wildtype glioblastoma (GBM). Median age (46.5 years) was significantly older than other circumscribed gliomas and younger than GBM. Dimensionality reduction with uniform manifold approximation and projection (UMAP) and hierarchical clustering confirmed a methylation signature distinct from known tumor types and methylation classes. DNA sequencing revealed recurrent mutations in *TP53* (57%), *BRAF* (26%), *NF1* (26%), and *NF2* (14%). *BRAF* V600E mutations were detected in 3/23 sequenced cases (13%). Copy number analysis revealed recurrent whole chromosome gains/losses with recurrent loss of chromosome 13 (28/31 cases, 90%). *CDKN2A/B* deletion (7/31, 6%) and *MGMT* promoter methylation (3/31, 10%) were mostly non-events. Most tumors showed features of a high-grade glioma, yet survival data showed significantly better overall survival compared to GBM ($p < 0.0001$). In summary, we describe a previously unrecognized group of adult-type gliomas characterized by a distinct DNA methylation signature and recurrent loss of chromosome 13.



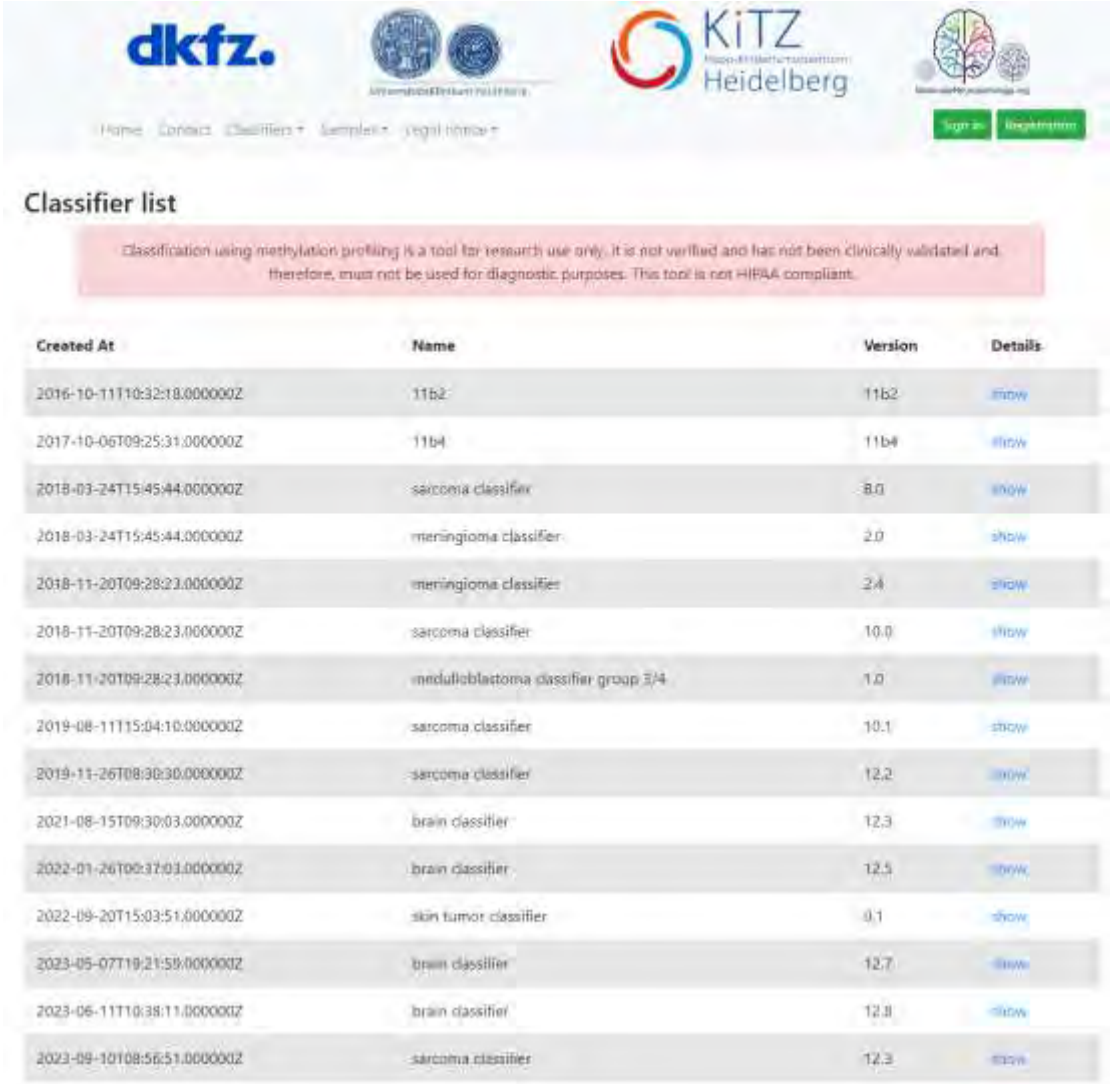




Challenges/limitations of methylation profiling

1. Expensive
2. No specific CPT code
3. Only available in a few large referral centers
4. Long turnaround time (2 weeks ~ months)
5. Requires high tumor concentration
6. 20 ~ 30% cases can’t be classified confidently

Future directions: Improve algorithm / classifier



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Future directions: Nanopore sequencing?

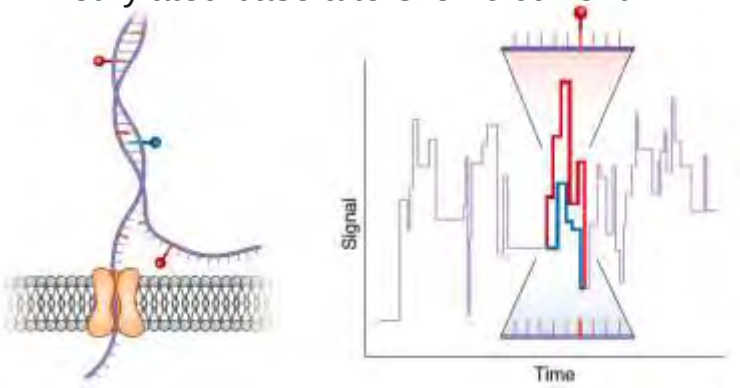
Advantages:

1. Directly detect methylated cytosines without bisulfite conversion
2. Rapid, real-time data generation
3. Relative low setup cost

Disadvantages:

1. Can only generate very sparse methylation profiles
2. Requires separate algorithms / classifiers

Methylated base alters ionic current



Schatz, M. Nanopore sequencing meets epigenetics. *Nat Methods* **14**, 347–348 (2017)

Future directions: Nanopore sequencing?

Article

Ultra-fast deep-learned CNS tumour classification during surgery

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Central nervous system tumours represent one of the most lethal cancer types, particularly among children. Primary treatment includes neurosurgical resection of the tumour, in which a delicate balance must be struck between maximizing the extent of resection and minimizing risk of neurological damage and comorbidity[1–3]. However, surgeons have limited knowledge of the precise tumour type prior to surgery. Current standard practice relies on preoperative imaging and intraoperative histological analysis, but these are not always conclusive and can occasionally be misleading[4]. Using rapid nanopore sequencing, a sparse methylation profile can be obtained during surgery[5]. Here we developed Sturgeon, a patient-agnostic transfer-learned neural network, to enable molecular subclassification of central nervous system tumours based on such sparse profiles. Sturgeon delivered intraoperative diagnoses within 40 minutes after starting sequencing in 45 out of 50 retrospectively acquired samples (obtaining from diagnosis of the other 5 samples). Furthermore, we demonstrated its applicability in real time during 25 surgeries, achieving a diagnostic turnaround time of less than 90 min. Of these, 18 (72%) diagnoses were correct and 7 did not reach the required confidence threshold. We conclude that machine-learned diagnosis based on low-cost intraoperative sequencing can assist neurosurgical decision-making, potentially preventing neurological comorbidity and avoiding additional surgeries.

The most common first-line treatment for central nervous system (CNS) tumours is neurosurgical resection of the tumour. An important factor in determining whether the risk of such aggressive intervention is acceptable is the tumour type. For instance, diffuse midline glioma with a mutation in histone H3 (refs. 1,2) invasion is unconditionally unable, indicating that surgery should primarily be aimed at acquisition of tumour tissue for diagnosis and preserving quality of life, rather than attempting complete resection[3]. Similarly, medulloblastoma show limited prognostic improvement between near-total and total resection, indicating that maximal resection is not necessarily preferable for these tumours[6]. However, radical resection is beneficial for other tumour types: in posterior fossa ependymoma type A and atypical teratoid rhabdoid tumour, a strategy of aiming for gross total resection should be followed, since this is an important prognostic factor[7–9]. Moreover, in CNS tumours in adults, the extent of resection matters: gross total resection has been reported to offer survival benefits for isocitrate dehydrogenase (IDH)-wild-type glioblastoma (the receptor tyrosine kinase (RTK) I and RTK II subtypes, but not for the mesenchymal subtype[10]. Similarly, in IDH-mutant astrocytoma, overall survival is negatively affected when gross total resection is not achieved[11]. The neurosurgical strategy thus depends on a precise and reliable diagnosis of the tumour.

Altered genome-wide DNA methylation patterns are highly characteristic of many tumour types and the examination of DNA methylation can reveal information about the origin and progression of tumours[12–14]. High-dimensional methylation profiles can be accurately assigned to a specific CNS subtype using machine learning approaches, in particular random forest classification[15,16]. Methylation arrays[17], in combination with the algorithm described by Capper et al.[18], are widely used in routine diagnostic practice. However, the turnaround time for obtaining array-based methylation profiles is in the order of several days and therefore incompatible with an intraoperative setting.

Current practice consists of preoperative imaging and intraoperative diagnosis achieved by rapid histological assessment of frozen tissue sections. However, this does not always result in a clear diagnosis, and the provisional frozen-section diagnosis is sometimes revised on the basis of post-operative tissue-based diagnostics. As a result, some patients require a second surgery, whereas others could in hindsight have been operated less radically.

Nanopore DNA sequencing has recently emerged as a method that enables ultra-rapid sequencing-based diagnosis[19,20]. Major advantages of nanopore sequencing include its low setup cost, small form factor and instant data availability. In addition, nanopore sequencing enables

Clinical Chemistry 70:1
250–260 (2024)

Cancer Diagnostics

Classification of Brain Tumors by Nanopore Sequencing of Cell-Free DNA from Cerebrospinal Fluid

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BACKGROUND: Molecular brain tumor diagnosis is usually dependent on tissue biopsies or resections. This can pose several risks associated with anesthesia or neurosurgery, especially for lesions in the brain stem or other difficult-to-reach anatomical sites. Apart from initial diagnosis, tumor progression, recurrence, or the acquisition of novel genetic alterations can only be proven by a biopsy.

METHODS: We employed Nanopore sequencing on cell-free DNA (cfDNA) from cerebrospinal fluid (CSF) and analyzed copy number variations (CNV) and global DNA methylation using a random forest classifier. We sequenced 129 samples with sufficient DNA. These samples came from 99 patients and encompassed 27 entities. Results were compared to clinical diagnosis and molecular analysis of tumor tissue, if available.

RESULTS: 110/129 samples were technically successful, and 50 of those contained detectable circulating tumor DNA (ctDNA) by CNV or methylation profiling. ctDNA was detected in samples from patients with progressive disease but also from patients without known residual disease. CNV plots showed diagnostic and prognostic alterations, such as *CDK6* amplifications in embryonal tumors with multilayered rosettes or Chr.1q gains and Chr.6q losses in posterior fossa group A ependymoma, respectively. Most CNV profiles mirrored the profiles of the respective tumor tissue. DNA methylation allowed exact classification of the tumor in 22/110 cases and led to incorrect classification in 2/110 cases. Only 5/50 samples with detected ctDNA contained tumor cells detectable through microscopy.

CONCLUSIONS: Our results suggest that Nanopore sequencing data of cfDNA from CSF samples may be a promising approach for initial brain tumor diagnostics and an important tool for disease monitoring.

Introduction

Central nervous system (CNS) tumors are very heterogeneous and can be classified into more than 100 different entities, according to the most recent 2021 World Health Organization guidelines (1). For treatment planning, it is essential to diagnose the exact type and subtype of the tumor. Here, the diagnosis heavily relies on the histological characteristics of the tumor. More recently, sequencing technologies and DNA methylation analyses have become valuable if not essential tools for diagnostics. Sequencing may identify diagnostic hallmarks or targetable alterations in a tumor. However, robust estimations on the molecular tumor entity are hard to obtain from sequencing alone, as tumor entities may not be characterized by specific single nucleotide variants or gene fusions. By global DNA methylation profiling, thousands of CpG sites within the genome are evaluated, resulting in a multidimensional fingerprint of the tumor. This signature can then be compared with reference databases to determine the highest similarity of the sample to a specific CNS tumor entity (2). As opposed to

Summary

1. Whole-genome DNA methylation profiling is a robust technique that utilizes machine learning to improve diagnostic accuracy and to identify new tumor types.

2. DNA methylation array is expensive, time-consuming, and not widely available.

3. Nanopore sequencing may potentially perform rapid methylation-based classification in a low-throughput setting

Thank you!

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