Methylation profiling – Pros and cons

H.K. Ng

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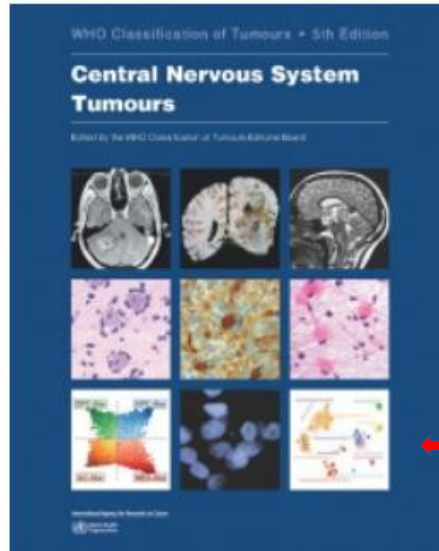
Ppt at http://www.acp.cuhk.edu.hk

Disclaimers

- Covers both adult and pediatric "type" tumors; the two are not totally separable
- Some opinions are personal ; you may not agree; colored by me being a pathologist and diagnostician
- Relate more to Asian than western situations
- Show some personal experience how we have used methylation, please excuse

About 50% of the entities in WHO 2021
have methylation as an essential or recommended
diagnostic feature

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WHO 2021 Classification and methylation profiling

Essential "for unresolved lesions" *	Essential Option "OR" #	Essential "AND" !	Desirable
Diffuse hemispheric glioma, H3 G34-mutant	Diffuse astrocytoma, *MYB*- or *MYBL1*-altered	Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC)	Astrocytoma, IDH-mutant
Astrocytoma, *MN1*-altered	Diffuse midline glioma, H3 K27 altered (of one of the subtypes of diffuse midline glioma)	High-grade astrocytoma with piloid features	Oligodendroglioma, IDH-mutant and 1p/19q-codeleted
Desmoplastic infantile astrocytoma (DIA) and Desmoplastic infantile ganglioglioma (DIG)	Infant-type hemispheric glioma	Posterior fossa group B (PFB) ependymoma	Glioblastoma, IDH-wild-type
Dysembryoplastic neuroepithelial tumor	Ganglioglioma	Diffuse pediatric-type high-grade (pHGG), H3 wt and IDH-wt (aligned with pHGG *RTK1*, pHGG *RTK2*, or pHGG *MYCN*)	Angiocentric glioma (aligned with diffuse glioma, *MYB*- or *MYBL1*-altered)
Papillary glioneuronal tumor	Posterior fossa group A (PFA) ependymoma	OR	Diffuse low-grade glioma, MAPK pathway-altered (Absence of molecular features or DNA methylation profiling suggestive of an alternative tumor type in which FGFR or *BRAF* abnormalities occur)
Rosette-forming glioneuronal tumor	Medulloblastoma, WNT-activated	Key molecular features: PDGFRA alterations, *EGFR* alteration or *MYCN*	Pleomorphic xanthoastrocytoma
Diffuse leptomeningeal glioneuronal tumor	Medulloblastoma, SHH-activated and *TP53*-wild-type		Subependymal giant cell astrocytoma
Central neurocytoma	Medulloblastoma, SHH-activated and *TP53*-mutant		Chordoid glioma
Extraventricular neurocytoma	Medulloblastoma, non-WNT/non-SHH		Myxoid glioneuronal tumor
Cerebellar liponeurocytoma	Meningioma: Suggestive histopathological features combined with one of the defined DNA methylation classes of meningioma		Supratentorial ependymoma, *ZFTA* fusion-positive
Myxopapillary ependymoma			Supratentorial ependymoma, *YAP1* fusion-positive
Subependymoma			Spinal ependymoma
Atypical teratoid/rhabdoid tumor			Spinal ependymoma, *MYCN*-amplified
Embryonal tumor with multilayered rosettes (ETMR)			Choroid plexus carcinoma
CNS neuroblastoma, FOXR2-activated			Pineoblastoma subtype
CNS tumor with *BCOR* internal tandem duplication			*CIC*-rearranged sarcoma
Pineal parenchymal tumor of intermediate differentiation			
Papillary tumor of the pineal region (confirmatory)			
Desmoplastic myxoid tumor of the pineal region, *SMARCB1*-mutant (confirmatory)			
Malignant melanotic nerve sheath tumor			
Cauda equina neuroendocrine tumor			
Primary intracranial sarcoma, *DICER1*-mutant			

Singh, Suri. NOP 2023

Epigenetics of tumors

- DNA methylation
- Histone modifications
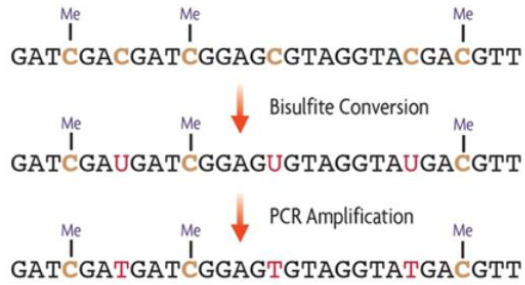- Remodeling of nucleosomes
- Non-coding RNA

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Basic assumptions

- Patterns of methylation (methylomes) reflect cells of origin
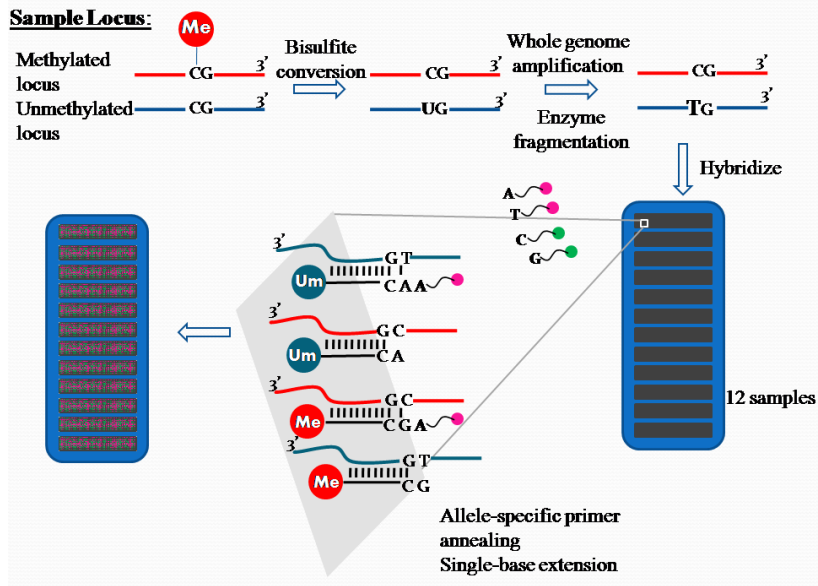- Methylation suppresses tumor suppressor genes in cancers

  Therefore, useful for tumor classification

- Histology can be observer-dependent

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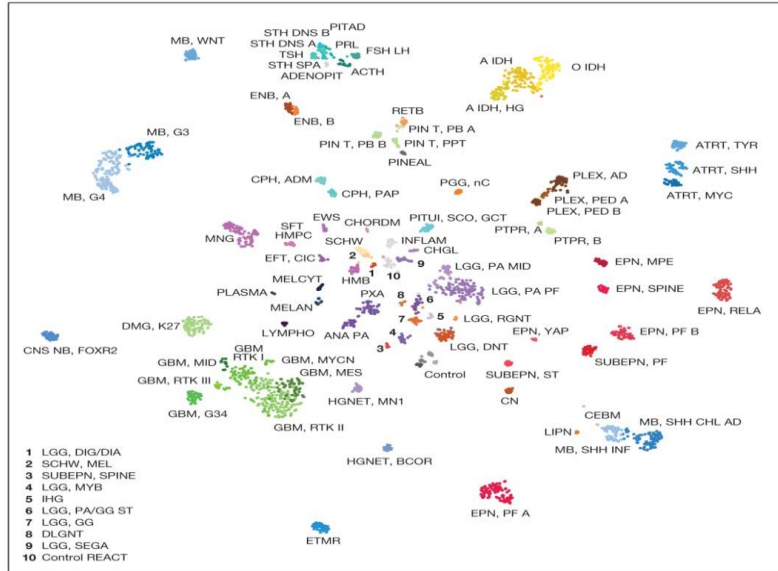Treatment by sodium bisulfite distinguishes between methylated and unmethylated CpG

Phoebe Lu

Signal intensities are processed to Illumina format files

Binary
IDAT
files
(1 for red,
 1 for green)

Capper *et al. Nature* **555**, 469–474 (2018)

Reference cohort of nearly 3,000 cases

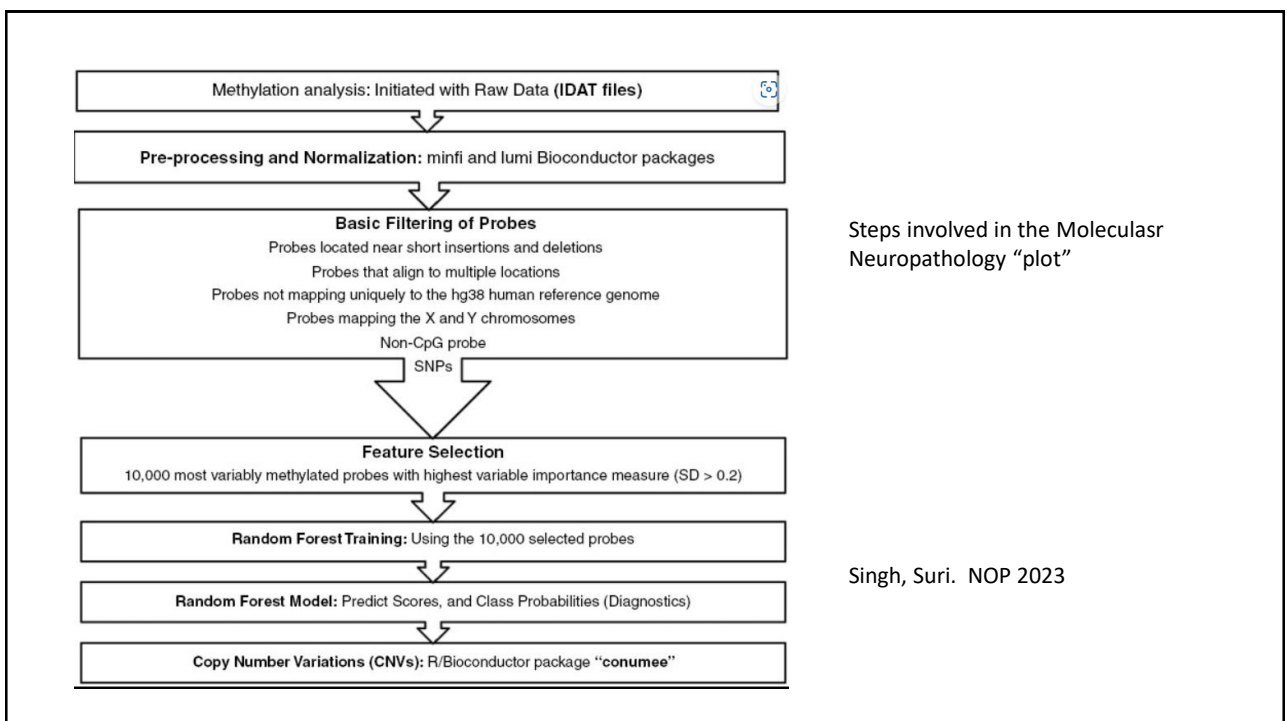## Welcome to MolecularNeuropathology.org - The platform for next generation neuropathology.

An updated version of the classifier handling EPICv2 chips (v12.8) is now available. Since, the majority of new uploads are with EPICv2, the new worklfow is the default. Please, use the workflow execution fields to analyse samples with other pipelines. The brain classifier v12.8 is at the moment the only one compatable with the EPICv2. A compatible version of the sarcoma classifier will be available soon. There is a new workflow with deactivated gender and adapted copy number generation (exclusion of ChrX and ChrY).

This website represents the access point for DNA methylation-based classification of central nervous system tumors. For the scientific background and interpretation of the data, please see Capper D, Jones DTW, Sill M, Hovestadt V et al., Nature. 2018 Mar 22; 555(7697):469-474.
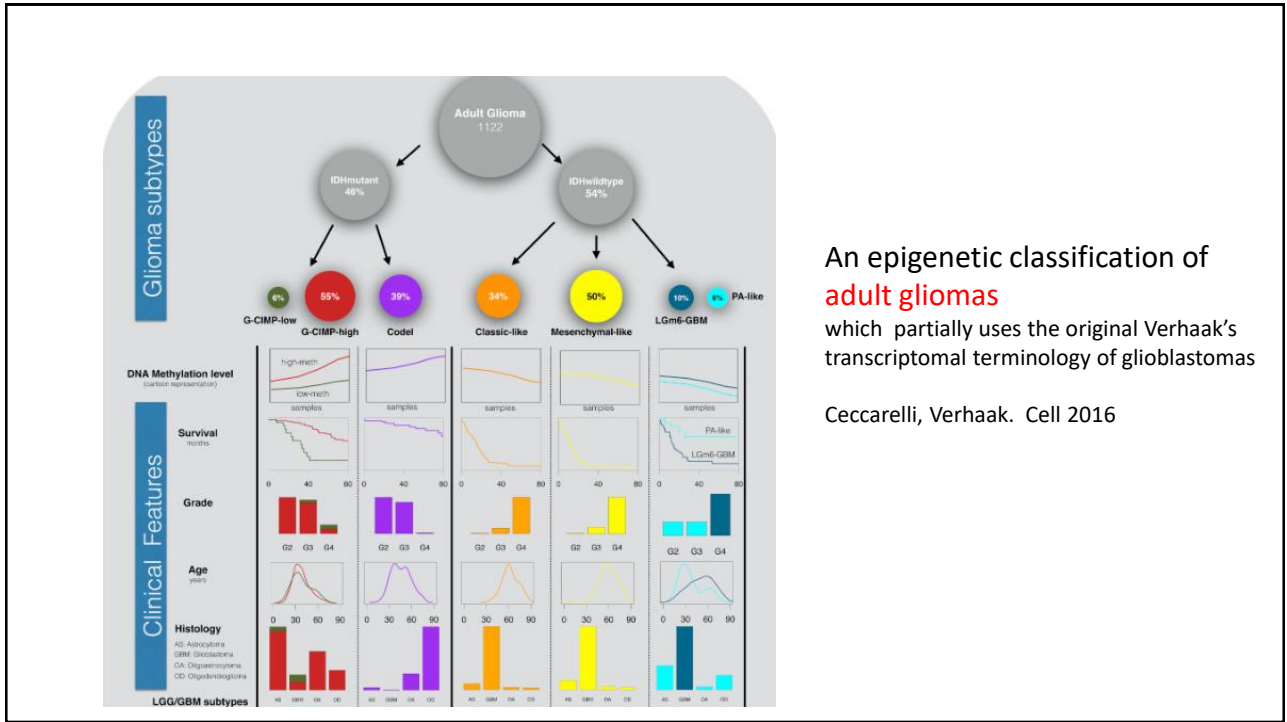
Upload statistic
Total cases: 121879
For classifier development: 91986

Involved parties
University Hospital Heidelberg
Neuropathology
Pediatric Oncology
Neurooncology
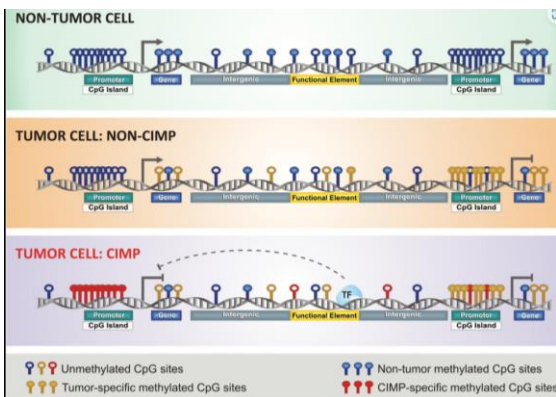Neurosurgery
Radiation Oncology
and Therapy

German Cancer Research Center (DKFZ)
Pediatric
Neurooncology
CCU Neuropathology

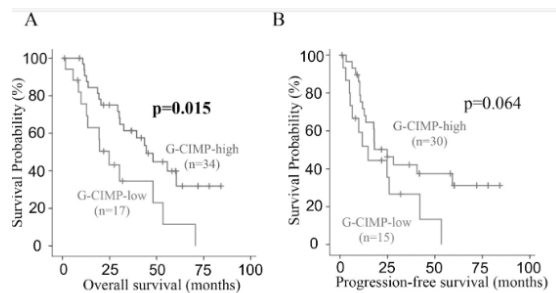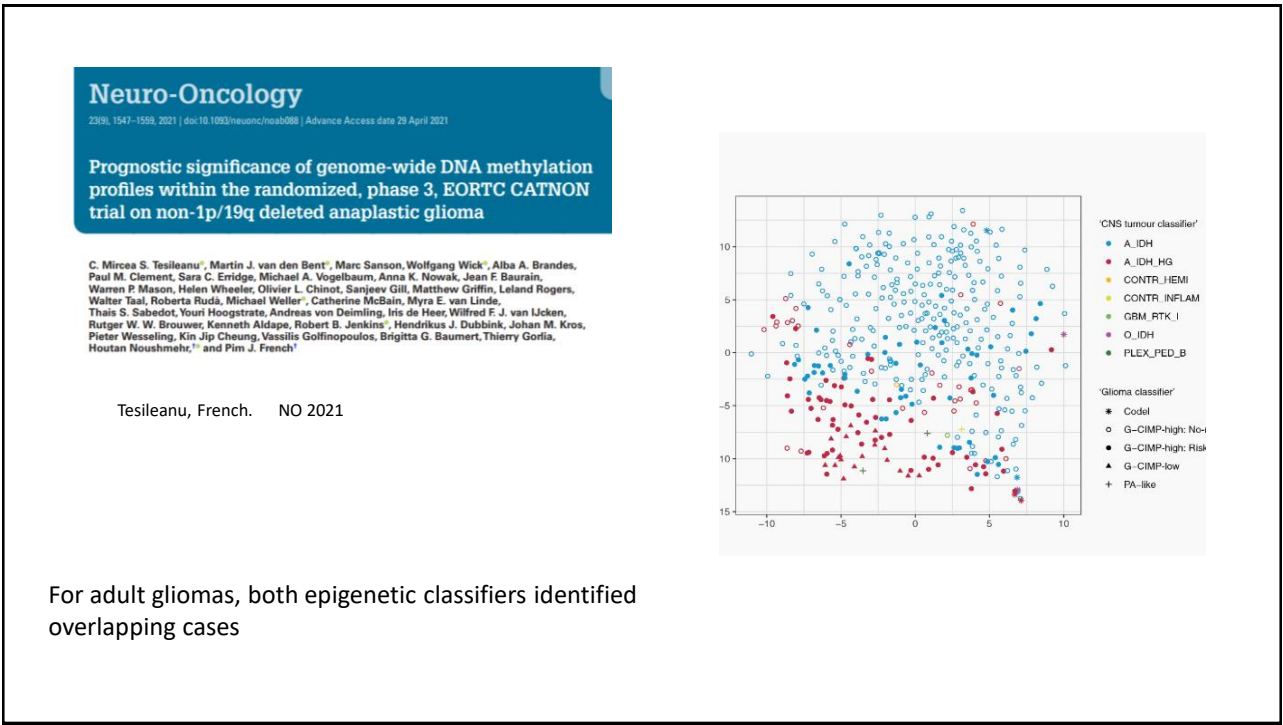Steps involved in the Moleculasr Neuropathology "plot"

Singh, Suri. NOP 2023

> Nat Commun. 2021 Jan 21;12(1):498. doi: 10.1038/s41467-020-20603-4.

Sarcoma classification by DNA methylation profiling

Christian Koelsche [# 1 2 3], Daniel Schrimpf [# 1 2], Damian Stichel [# 2], Martin Sill [# 4 5],
Felix Sahm [1 2], David E Reuss [1 2], Mirjam Blattner [4 6], Barbara Worst [4 6 7], Christoph E Heilig [8],
Katja Beck [8 9], Peter Horak [8], Simon Kreutzfeldt [8], Elke Paff [4 6 7], Sebastian Stark [4 6 7],
Pascal Johann [4 6 7], Florian Selt [4 7 10], Jonas Ecker [4 7 10], Dominik Sturm [4 6 7],
Kristian W Pajtler [4 5 7], Annekathrin Reinhardt [1 2], Annika K Wefers [1 2], Philipp Sievers [1 2],
Azadeh Ebrahimi [2], Abigail Suwala [1 2], Francisco Fernández-Klett [1 2], Belén Casalini [2],
Andrey Korshunov [1 2], Volker Hovestadt [11 12], Felix K F Kommoss [3], Mark Kriegsmann [3],
Matthias Schick [13], Melanie Bewerunge-Hudler [13], Till Milde [4 7 10], Olaf Witt [4 7 10],
Andreas E Kulozik [4 7], Marcel Kool [4 5], Laura Romero-Pérez [14], Thomas G P Grünewald [14],
Thomas Kirchner [15], Wolfgang Wick [16 17], Michael Platten [18 19], Andreas Unterberg [20],
Matthias Uhl [21 22], Amir Abdollahi [21 22 23 24], Jürgen Debus [21 22 23 24], Burkhard Lehner [25],
Christian Thomas [26], Martin Hasselblatt [26], Werner Paulus [26], Christian Hartmann [27],
Ori Staszewski [28 29], Marco Prinz [28 30 31], Jürgen Hench [32], Stephan Frank [32],
Yvonne M H Versleijen-Jonkers [33], Marije E Weidema [33], Thomas Mentzel [34], Klaus Griewank [35],

Sarcoma classification by DNA methylation profiling

Fig. 1: Establishing the DNA methylation-based sarcoma reference cohort.

More difficult with cancers with an anatomical boundary as a critical diagnostic criteria (e.g. muscularis propria, muscularis mucosae, basement membrane) ?

Isocitrate dehydrogenase (IDH) mutation
- Oncogenic metabolite D2-HG competes with histone demyethylase and leads to increased genomic methylation

Miller, Wen. NO 2022

An epigenetic classification of
adult gliomas
which partially uses the original Verhaak's
transcriptomal terminology of glioblastomas

Ceccarelli, Verhaak. Cell 2016

Use of G-cimp

Malta, Noushmehr. NO 2018

IDH mutant Grader 4 astrocytomas

Wong, Noushmehr, Ng. Modern Pathology 2021

Supplementary Figure 1

IDHwt glioblastomas

The Ceccarelli epigenetic classification can be used quite well for clinicopathologic correlation

Liu, Noushmehr, Ng. Brain Pathology 2022

Neuro-Oncology
23(9), 1547–1559, 2021 | doi:10.1093/neuonc/noab088 | Advance Access date 29 April 2021

Prognostic significance of genome-wide DNA methylation profiles within the randomized, phase 3, EORTC CATNON trial on non-1p/19q deleted anaplastic glioma

C. Mircea S. Tesileanu, Martin J. van den Bent, Marc Sanson, Wolfgang Wick, Alba A. Brandes, Paul M. Clement, Sara C. Erridge, Michael A. Vogelbaum, Anna K. Nowak, Jean F. Baurain, Warren P. Mason, Helen Wheeler, Olivier L. Chinot, Sanjeev Gill, Matthew Griffin, Leland Rogers, Walter Taal, Roberta Rudà, Michael Weller, Catherine McBain, Myra E. van Linde, Thais S. Sabedot, Youri Hoogstrate, Andreas von Deimling, Iris de Heer, Wilfred F. J. van IJcken, Rutger W. W. Brouwer, Kenneth Aldape, Robert B. Jenkins, Hendrikus J. Dubbink, Johan M. Kros, Pieter Wesseling, Kin Jip Cheung, Vassilis Golfinopoulos, Brigitta G. Baumert, Thierry Gorlia, Houtan Noushmehr, and Pim J. French

Tesileanu, French.   NO 2021

For adult gliomas, both epigenetic classifiers identified overlapping cases

Uploading of IDAT files
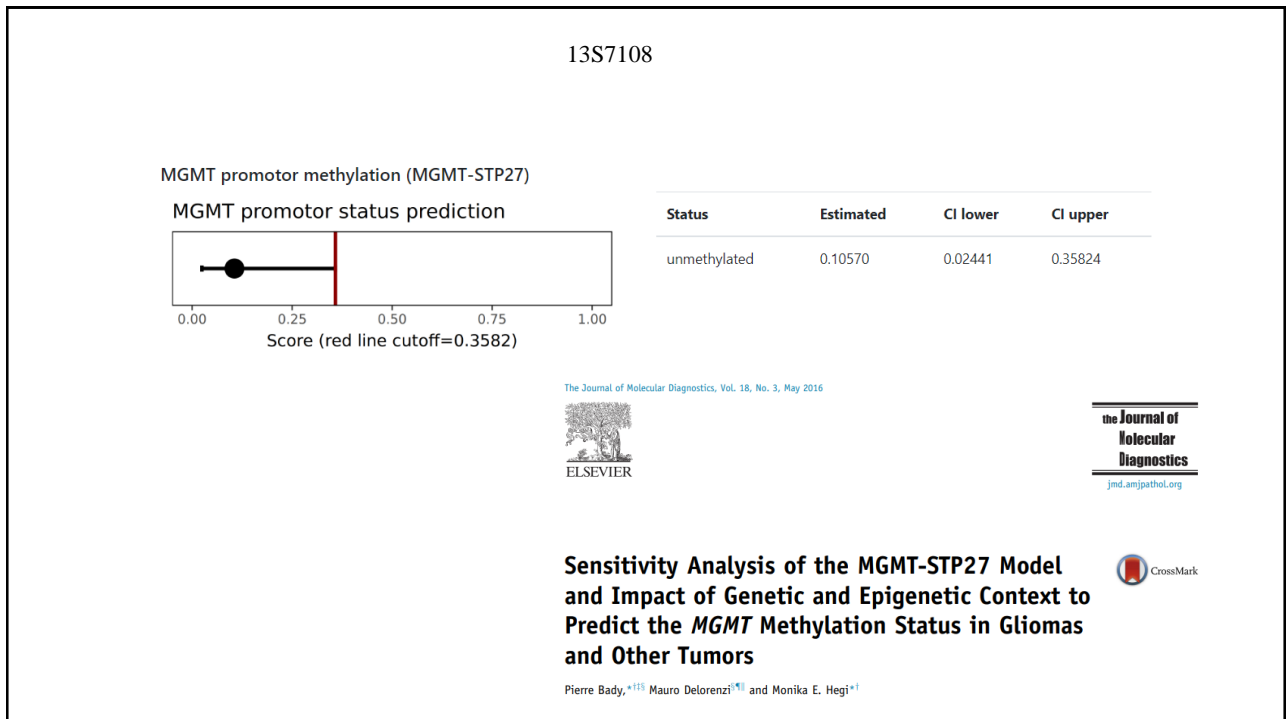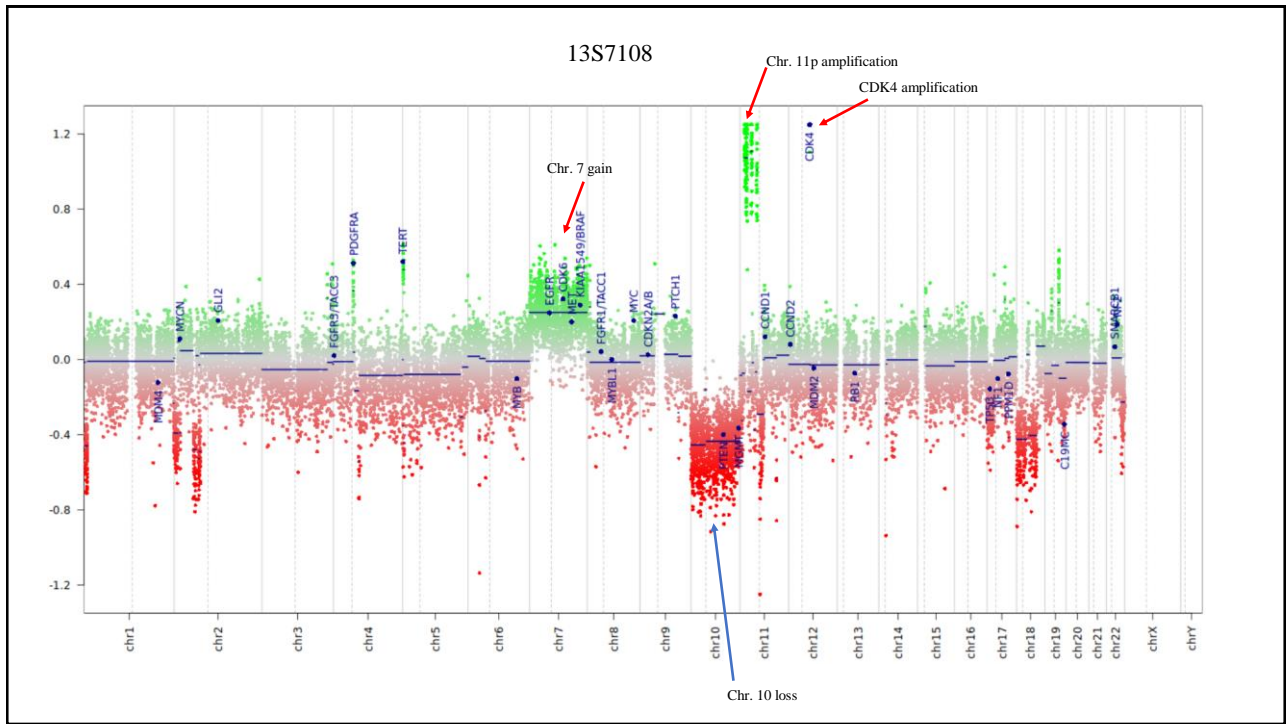to DKFZ Classifier website

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13S7108

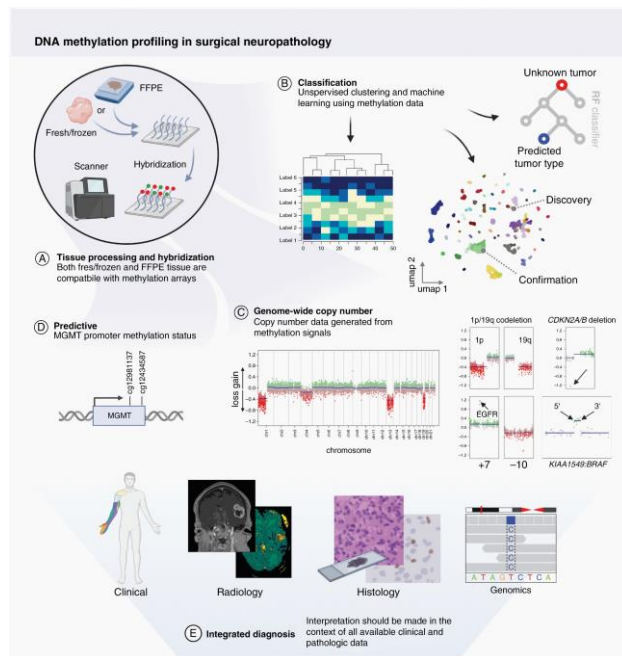Classifier prediction (Version 12.8 of the brain classifier; Version: 12.8)

Methylation classes (Highest level >= 0.3, lower levels >= 0.1, all remaining reference groups)	Calibrated score	Interpretation	
Adult-type diffuse gliomas	0.98263	match	✔
Glioblastoma, IDH-wildtype	0.98180	match	●
Glioblastoma, IDH-wildtype, RTK2 type	0.92917	match	●
MC Glioblastoma, IDH-wildtype, RTK2 subtype	0.92917	match	●

Legend: ✔Match (score >= 0.9) ✘No match (score &lt: 0.9): possibly still relevant for low tumor content and low DNA quality cases. ●Match to MC family member (score >= 0.9)

Description:

The "mc Glioblastoma, IDH-wildtype, RTK2 subtype" is comprised of diffuse, astrocytic gliomas that are IDH-wildtype and H3-wildtype and have one or more of the following histological or genetic features: microvascular proliferation, necrosis, TERT promoter mutation, EGFR gene amplification, +7/−10 chromosome copy-number changes (CNS WHO grade 4). They also very commonly (~90%) harbor TP53 mutations and CDKN2A/B deletions (~70%). They can manifest in patients of any age but preferentially affects older adults, with peak incidence in patients aged 55–85 years. The RTK2 subtype harbours a high frequency of EGFR amplification (~60%). Expression profiles often resemble the 'Proneural' subgroup according to the TCGA classification. Expression profiles often resemble the 'Classical' subgroup according to the TCGA classification.

13S7108

Chr. 11p amplification

CDK4 amplification

Chr. 7 gain

Chr. 10 loss

13S7108

MGMT promotor methylation (MGMT-STP27)

MGMT promotor status prediction

Score (red line cutoff=0.3582)

Status	Estimated	CI lower	CI upper
unmethylated	0.10570	0.02441	0.35824

Sensitivity Analysis of the MGMT-STP27 Model and Impact of Genetic and Epigenetic Context to Predict the *MGMT* Methylation Status in Gliomas and Other Tumors

CrossMark

Pierre Bady,*†‡§ Mauro Delorenzi§¶‖ and Monika E. Hegi*†

DNA methylation profiling in surgical neuropathology

Pratt, Sahm, Aldape
NO 2021

Methylation profiling – the add ons

- MGMT status
- 1p19q status
- Gene copy variation of some genes
- chromosome changes (eg +7/-10)
- Selected fusion genes ?

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Figure 1: Establishing the DNA methylation-based CNS tumour reference cohort.
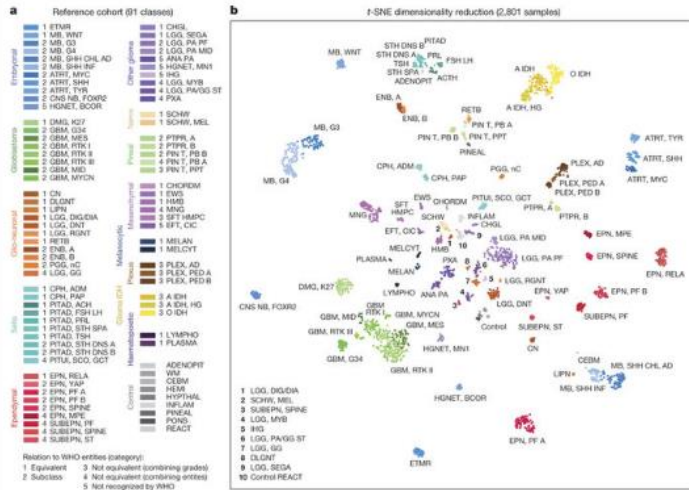
The tSNE plot of Capper 's by dimensional reduction

Capper D et al. Nature 2018

Methylation profiling Of Adult gliomas

Wong, Ng
Modern Pathology 2021
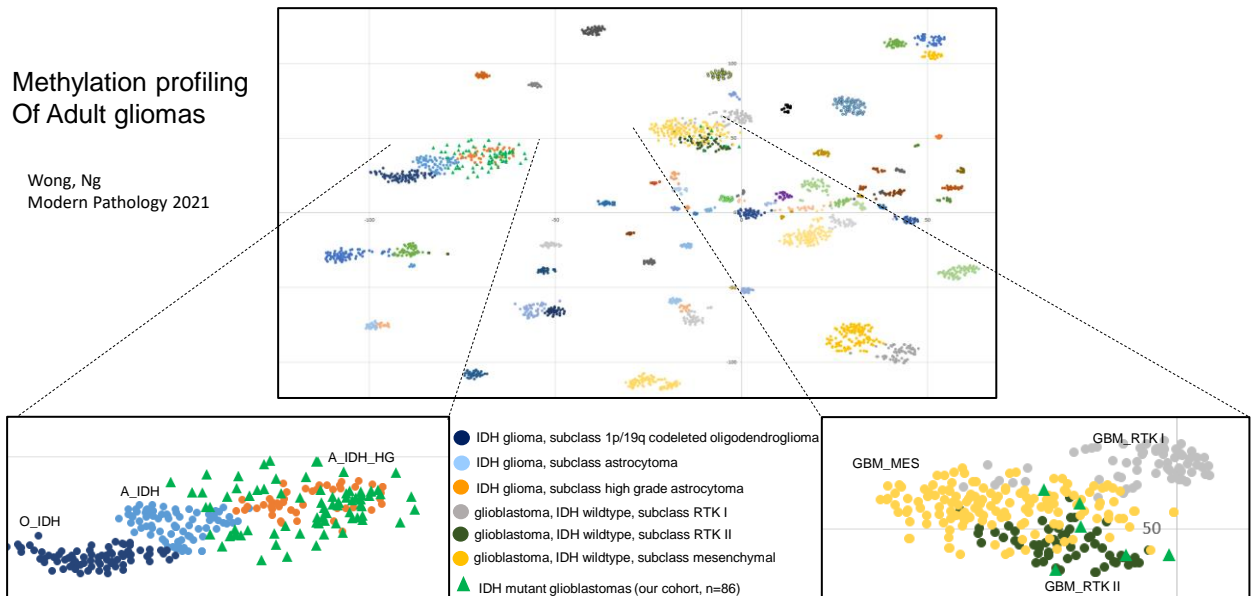
Figure 1. Unsupervised clustering of reference cohort samples and 85 IDH mutant glioblastomas using t-SNE dimensionality reduction. The reference cohort of the DKFZ CNS tumor classifier includes 82 tumour and 9 non-tumour classes and they are shown as circles of different colors. The 85 primary IDH mutant glioblastomas of our cohort clustered mainly to the (I) IDH mutant high grade astrocytomas; (2) glioblastoma, IDH wildtype, subclass RTK II and (3) subclass mesenchymal (green triangles). Mutations of IDH in our samples were tested and confirmed by independent PCR and sanger sequencing.

Pediatric H3 wt high grade gliomas
Hong, Ng. Laboratory Investigation 2022

HGG of adolescents and young adults

Shi, Ng. NAN 2022

WHO Classification 2021
Glial and glio-neuronal tumors

WHO Blue Book 2021
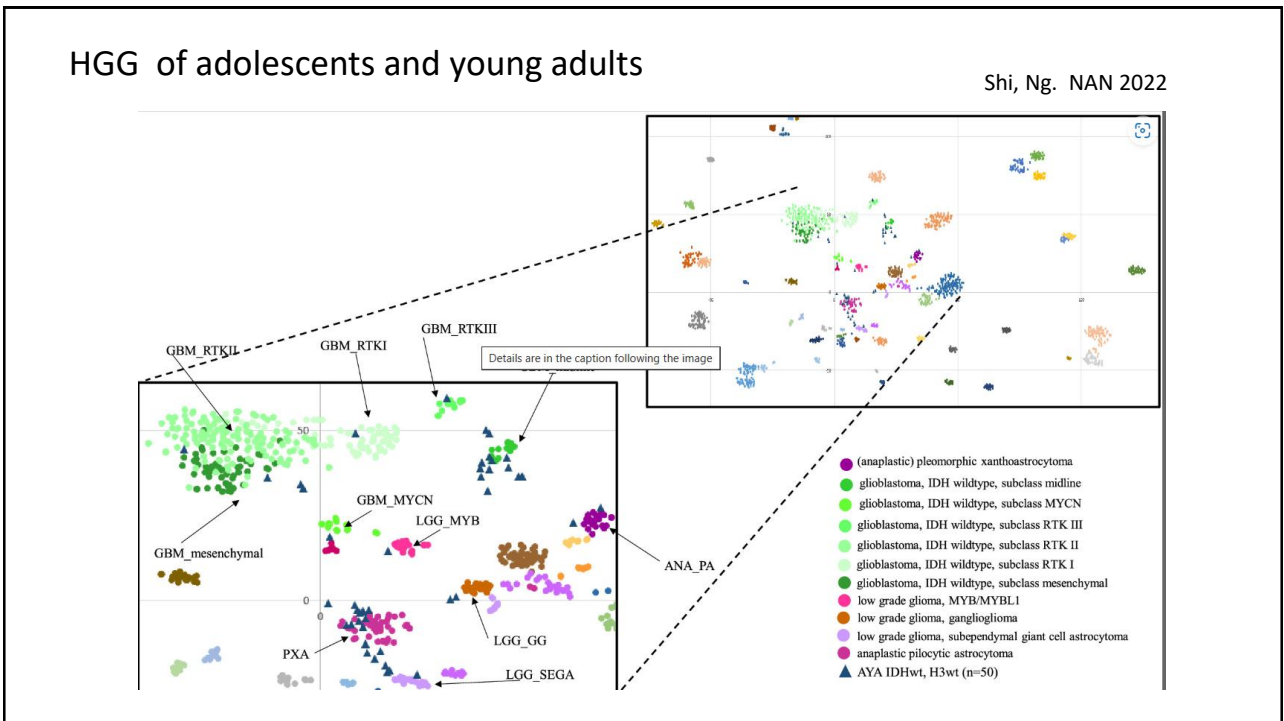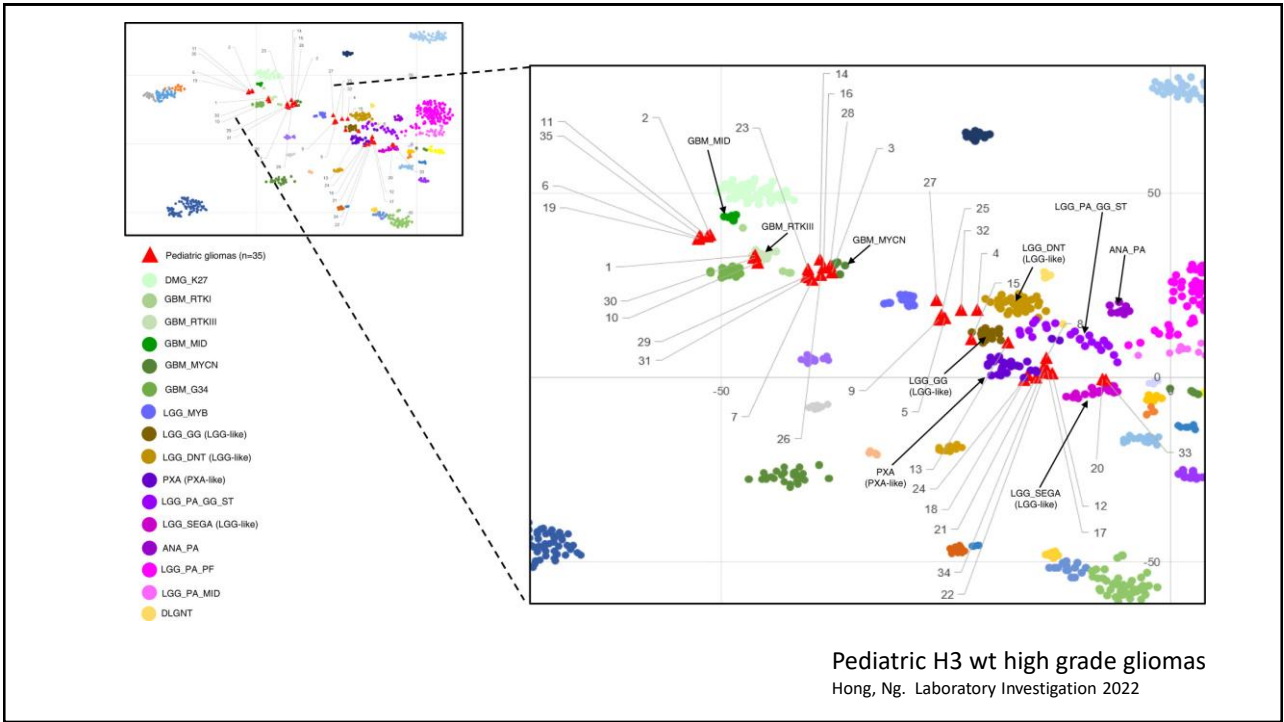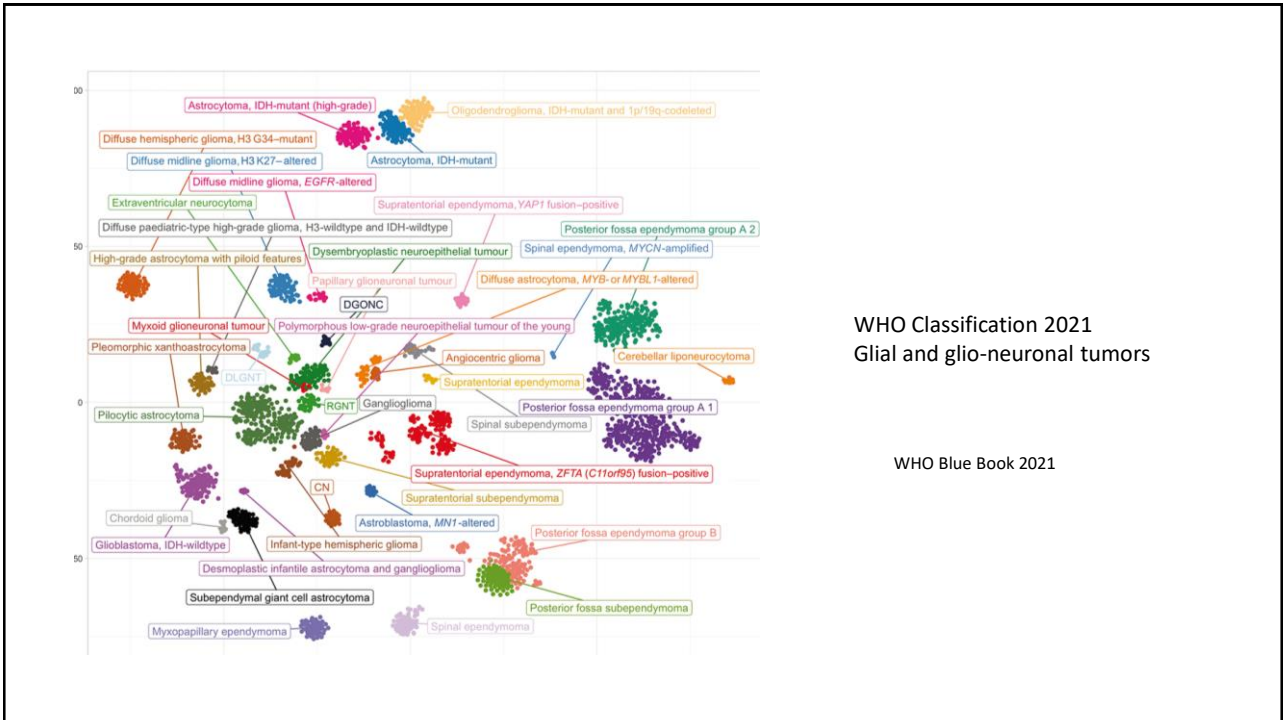
Advantages of methylation profiling

- Clarifying diagnosis – increase precision of diagnosis
- An uniform methodology – Illumina
- Discovery of new tumor entities
- Molecular grouping of medulloblastoma (limitations of transcriptomic methods eg nanostring assay)
- Fine stratification/ characterization of known entities (examples : meningiomas, pineal gland tumors, "PNET")

Fine stratification of meningiomas

THE LANCET
Oncology
Volume 18, Issue 5, May 2017, Pages 682-694

Articles

DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis

Felix Sahm MD [a,b], Daniel Schrimpf PhD [a], Damian Stichel PhD [b], David T W Jones PhD [c], Thomas Hielscher MSc [d], Sebastian Schefzyk MSc [a], Konstantin Okonechnikov PhD [c], Christian Koelsche MD [a,b], David E Reuss MD [a,b], David Capper MD [a,b], Dominik Sturm MD [c,i], Hans-Georg Wirsching MD [l], Anna Sophie Berghoff MD [m], Peter Baumgarten MD [o], Annekathrin Kratz MD [a,b], Kristin Huang MD [a,b], Annika K Wefers MD [a,b], Volker Hovestadt PhD [f], Martin Sill PhD [d], Hayley P Ellis BSc [p]...Prof Andreas von Deimling MD [a,b]
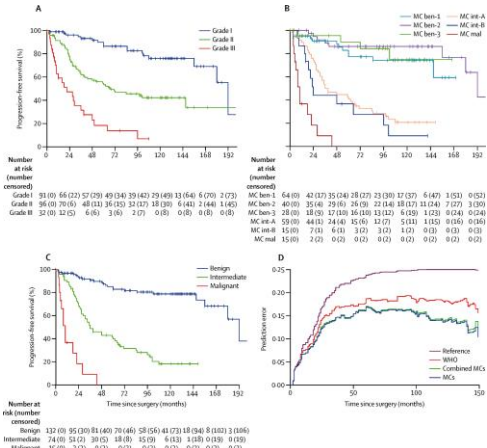
Fine characterization of existing tumors – pineal gland tumors

ORIGINAL PAPER

Clinical and molecular heterogeneity of pineal parenchymal tumors: a consensus study

Anthony P. Y. Liu[1,2] · Bryan K. Li[3,4,5] · Elke Pfaff[6,7,8] · Brian Gudenas[2] · Alexandre Vasiljevic[9,10] · Brent A. Orr[11] · Christelle Dufour[12,13] · Matija Snuderl[14,15] · Matthias A. Karajannis[16] · Marc K. Rosenblum[17] · Eugene I. Hwang[18] · Ho-Keung Ng[19] · Jordan R. Hansford[20] · Alexandru Szathmari[21] · Cécile Faure-Conter[22] · Thomas E. Merchant[23] · Max Levine[16] · Nancy Bouvier[16] · Katja von Hoff[24] · Martin Mynarek[25] · Stefan Rutkowski[25] · Felix Sahm[6,26,27] · Marcel Kool[6,28,29] · Cynthia Hawkins[4,5,30] · Arzu Onar-Thomas[31] · Giles W. Robinson[1] · Amar Gajjar[1] · Stefan M. Pfister[6,8,28] · Eric Bouffet[3] · Paul A. Northcott[2] · David T. W. Jones[6,7] · Annie Huang[3,4,5,32]

Liu, Huang. Acta Neuropath 2021
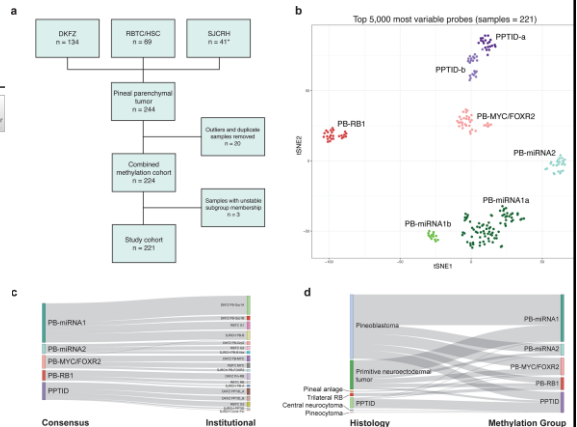
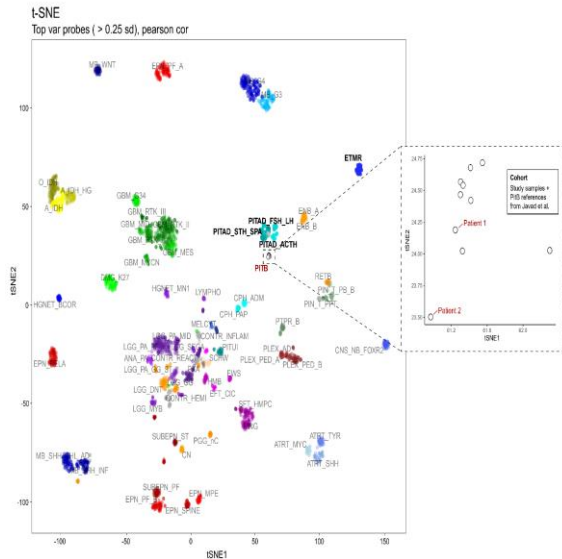Fine characterization of known rare tumors – pituitary blastomas

Expanding the clinical and molecular spectrum of pituitary blastoma

Anthony Pak-Yin Liu[1,2] · Kay Ka-Wai Li[3] · Chit Chow[3] · Shing Chan[1] · Alex Wing-Kwan Leung[2,4] ·
Matthew Ming-Kong Shing[2] · Ka-Fai To[3] · Danny Tat-Ming Chan[5] · Godfrey Chi-Fung Chan[1,2] · Ho-Keung Ng[3]

Liu, Ng. Acta Neuropath 2022


Discovery of new entities, recent examples

- High grade astrocytoma with piloid features (WHO entity)
- Neuroepithelial tumors with PATZ fusion
- Neuroepithelial tumors with PLAG1 fusion
- Oligosarcoma
- Glioneuronal tumor with ATRX alteration, kinase fusion and anaplastic features (GTAKA)
- More

Methylation for precise diagnosis

- Probably more useful in pediatric type brain tumors
- Adult brain tumors less useful, especially for the common diffuse gliomas
- Extremely useful for medulloblastoma subgrouping where the other methodologies used, IHC or nanostring, have limitations
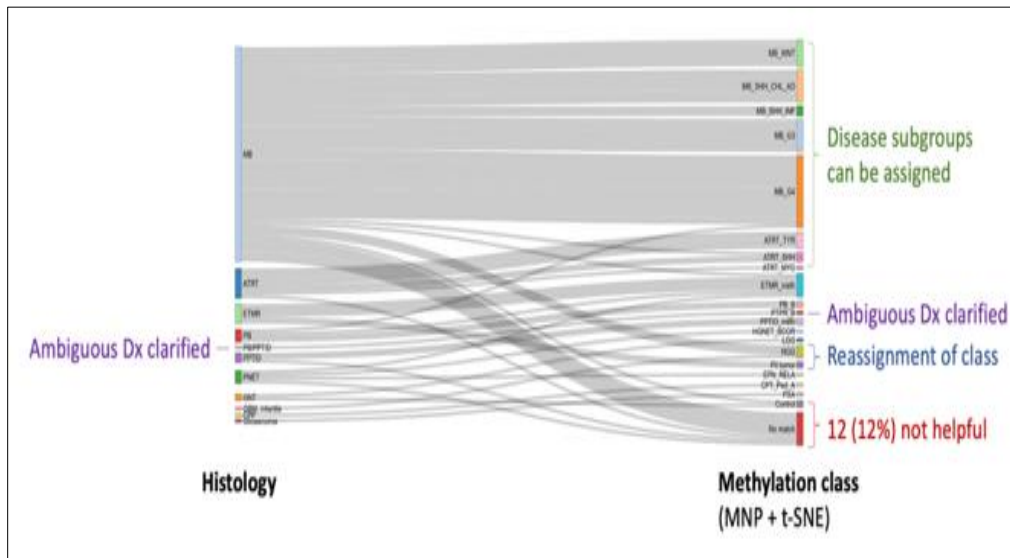- Extremely useful or a must for the rarer tumors

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Methylation profiling as a routine diagnostic tool – many studies
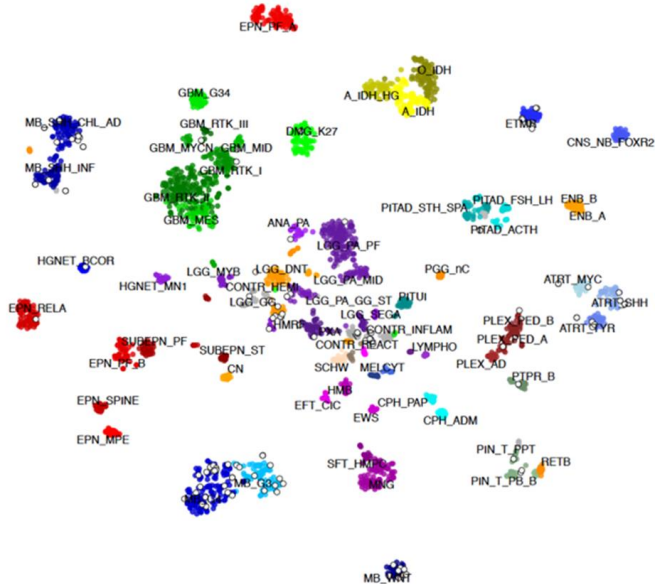
Series of 94 pediatric tumors

Tam, Ng, Liu. Cancers 2023

Calibrated score >9 : 65%
Calibrated score >6 : 75%
Informative with tSNE : 88%

Tam, Ng, Liu. Cancers 2023

Methylation profiling as a diagnostic tool - issues

- Cost
- Time and batch issues - ?? nanopore sequencing
- ? Grading
- Discrepancy with conventional testing, e.g. MGMT, 1p19q
- ? Fusion
- Not used in clinical trials yet
- Classifier not sync with WHO entities and nomenclature –significance of subclasses
- New versions and entities in the Classifier
- Actual case data of subsequent versions not available
- Scores >0.9 not always, what of scores of 0.5-0.9 ?
- Not pathway- or mechanism –related
- Medico-legal challenges

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Slide 1:

Robust methylation-based classification of brain tumours using nanopore sequencing

Luis P. Kuschel, Jürgen Hench, Stephan Frank, Ivana Bratic Hench, Elodie Girard, Maud Blanluet, Julien Masliah-Planchon, Martin Misch, Julia Onken, Marcus Czabanka ... See all authors ⌄

NAN 2022

Check for updates

Rapid-CNS^2: rapid comprehensive adaptive nanopore-sequencing of CNS tumors, a proof-of-concept study

Areeba Patel[1,2] · Helin Dogan[1,2] · Alexander Payne[3] · Elena Krause[1] · Philipp Sievers[1,2] · Natalie Schoebe[1,2] · Daniel Schrimpf[1,2] · Christina Blume[1,2] · Damian Stichel[1,2] · Nadine Holmes[3] · Philipp Euskirchen[4] · Jürgen Hench[5] · Stephan Frank[5] · Violaine Rosenstiel-Goidts[6] · Miriam Ratliff[7] · Nima Etminan[7] · Andreas Unterberg[8] · Christoph Dieterich[9] · Christel Herold-Mende[8] · Stefan M. Pfister[10,11,12] · Wolfgang Wick[14,15] · Matthew Loose[3] · Andreas von Deimling[1,2] · Martin Sill[10,11] · David T. W. Jones[10,13] · Matthias Schlesner[16] · Felix Sahm[1,2,10]🟢

Acta Neruropathologica 2022

Nanopore sequencing vastly reduces turnaround time

**Slide 2:**

Methylation part of the paper

1,182 tumors

21% could not be assigned a class (including tSNE)

2% of tumors classified as normal tissues

67.8% concordant with the histological (WHO) diagnoses

Refinement in diagnoses in 50%

Clinically relevant discrepancy in 5% (more in other series)

Most common discrepancy being histological APA or GBM assigned by methylation to PA, GG Or MYB/MYBL

Fig. 3: Landscape of DNA methylation classes and levels of concordance with WHO-based diagnosis.

From: Multiomic neuropathology improves diagnostic accuracy in pediatric neuro-oncology
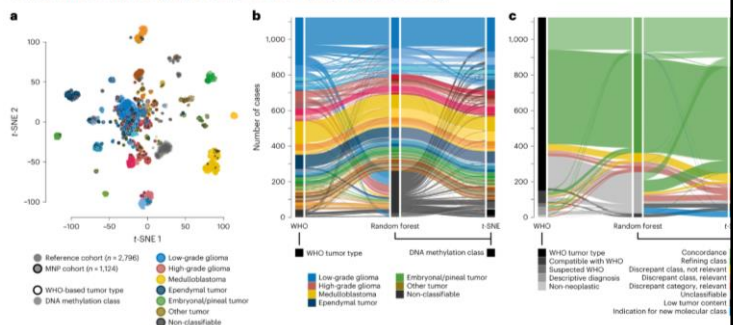
DKFZ's own experience of pediatric brain tumors
Sturm, Jones. Nature Medicine 2023

Occasional updates may be required for either inclusion of new tumor classes or subtle changes of the EPIC array probe composition that may occur in a new batch. Older version will remain availab

Classification using methylation profiling is a tool for research use only, it is not verified and has not been clinically validated and, therefore, must not be used for diagnostic purposes. This tool is not HIPAA compliant.

©MolecularNeuroPathology.org 2023 (Impressum) - Version 6.9.2 (Built with Bootstrap framework)
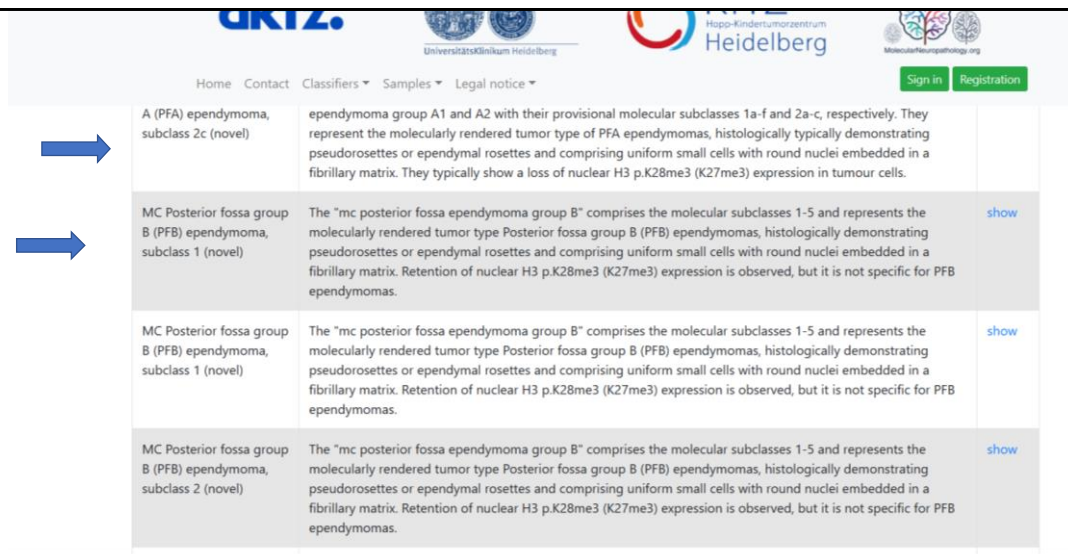
DKFZ Classifier website

Issues as a regular diagnostic tool

- Need for relatively high workload
- Time for results – difficulty of communication when dx changed
- ? Normal tissue ? Inflamed tissue as diagnoses
- Difficulty of communication with "undiagnosable" with patients and clinicians
- Expertise required if tSNE is needed - most studies used
- Gene fusions eg BRAF detection not always feasible
- Terminology not fully sync with WHO classifications

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2018-03-24T15:45:44.000000Z	meningioma classifier	2.0	show
2018-11-20T09:28:23.000000Z	meningioma classifier	2.4	show
2018-11-20T09:28:23.000000Z	sarcoma classifier	10.0	show
2018-11-20T09:28:23.000000Z	medulloblastoma classifier group 3/4	1.0	show
2019-08-11T15:04:10.000000Z	sarcoma classifier	10.1	show
2019-11-26T08:30:30.000000Z	sarcoma classifier	12.2	show
2021-08-15T09:30:03.000000Z	brain classifier	12.3	show
2022-01-26T00:37:03.000000Z	brain classifier	12.5	show
2022-09-20T15:03:51.000000Z	skin tumor classifier	0.1	show
2023-05-07T19:21:59.000000Z	brain classifier	12.7	show
2023-06-11T10:38:11.000000Z	brain classifier	12.8	show

It is unclear how many cases are in the cohorts of the newer versions

A (PFA) ependymoma, subclass 2c (novel)	ependymoma group A1 and A2 with their provisional molecular subclasses 1a-f and 2a-c, respectively. They represent the molecularly rendered tumor type of PFA ependymomas, histologically typically demonstrating pseudorosettes or ependymal rosettes and comprising uniform small cells with round nuclei embedded in a fibrillary matrix. They typically show a loss of nuclear H3 p.K28me3 (K27me3) expression in tumour cells.	
MC Posterior fossa group B (PFB) ependymoma, subclass 1 (novel)	The "mc posterior fossa ependymoma group B" comprises the molecular subclasses 1-5 and represents the molecularly rendered tumor type Posterior fossa group B (PFB) ependymomas, histologically demonstrating pseudorosettes or ependymal rosettes and comprising uniform small cells with round nuclei embedded in a fibrillary matrix. Retention of nuclear H3 p.K28me3 (K27me3) expression is observed, but it is not specific for PFB ependymomas.	show
MC Posterior fossa group B (PFB) ependymoma, subclass 1 (novel)	The "mc posterior fossa ependymoma group B" comprises the molecular subclasses 1-5 and represents the molecularly rendered tumor type Posterior fossa group B (PFB) ependymomas, histologically demonstrating pseudorosettes or ependymal rosettes and comprising uniform small cells with round nuclei embedded in a fibrillary matrix. Retention of nuclear H3 p.K28me3 (K27me3) expression is observed, but it is not specific for PFB ependymomas.	show
MC Posterior fossa group B (PFB) ependymoma, subclass 2 (novel)	The "mc posterior fossa ependymoma group B" comprises the molecular subclasses 1-5 and represents the molecularly rendered tumor type Posterior fossa group B (PFB) ependymomas, histologically demonstrating pseudorosettes or ependymal rosettes and comprising uniform small cells with round nuclei embedded in a fibrillary matrix. Retention of nuclear H3 p.K28me3 (K27me3) expression is observed, but it is not specific for PFB ependymomas.	show

Novel subtypes are introduced in new versions but the details of these cases which formulate these subtypes are not available

Correlation between WHO 2021 and DKFZ Classifier classification

Wenger, Caren. Cancers 2022
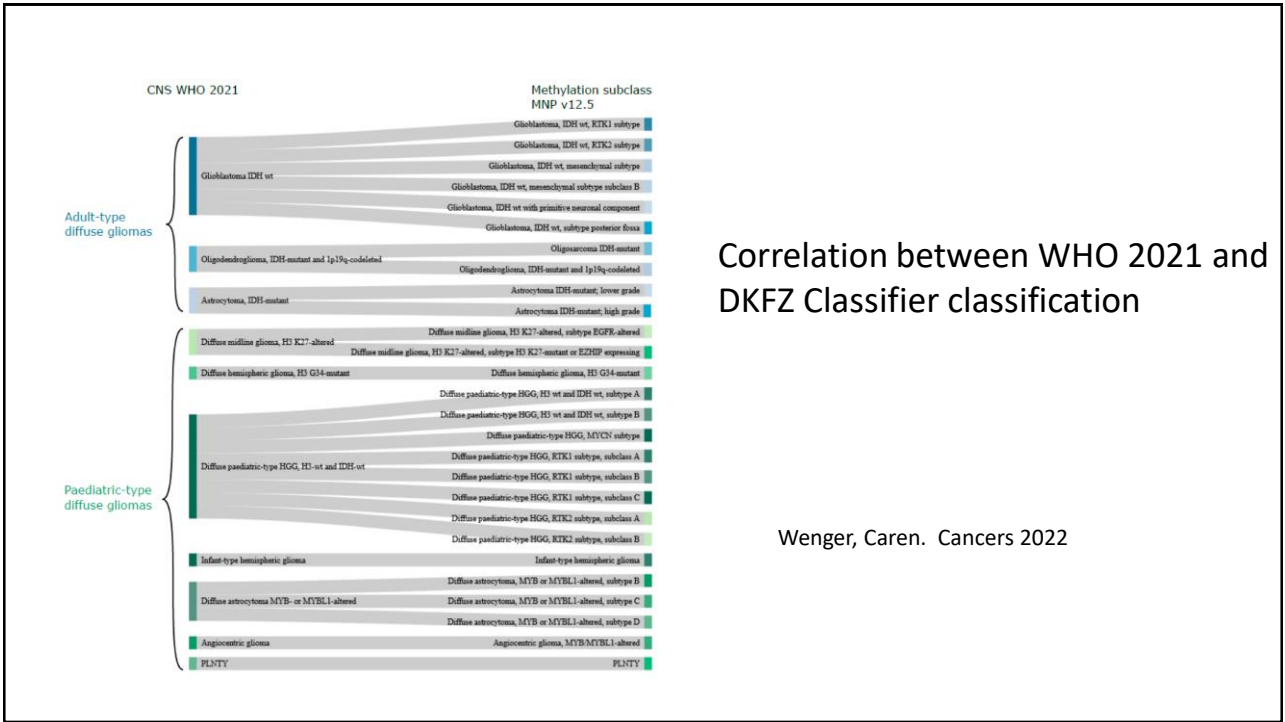
The four levels of classification at the DKFZ Classifier

Table 1. The four levels of hierarchy according to v12.5 of the MNP classifier for three cases with different types of diffuse gliomas. RTK—receptor tyrosine kinase, wt—wildtype.

| Hierarchy	Case 1	Case 2	Case 3
Superfamily	Adult-type diffuse gliomas	Adult-type diffuse gliomas	Paediatric-type diffuse high-grade gliomas
Family	Glioblastoma, IDH-wt	Diffuse glioma, IDH mutant	Diffuse paediatric-type high-grade glioma, H3-wt and IDH-wt
Class	Glioblastoma, IDH-wt, RTK1 type	Diffuse glioma, IDH-mutant and 1p19q codeleted [oligodendroglial type]	Diffuse paediatric-type high-grade glioma, RTK1 subtype
Subclass	Glioblastoma, IDH-wt, RTK1 subtype	Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	Diffuse paediatric-type high-grade glioma, RTK1 subtype, subclass A

Wenger, Caren. Cancers 2022

Methylation - other technical issues

- Low tumor content – diagnoses of "control tissues" or lower grade tumors
- Amount of DNA, Quality of DNA
- The Heidelberg group formed a company for methylation profiling
- Are there alternative methods of clustering, e.g. RNAseq

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Integrated approach to brain tumor diagnosis
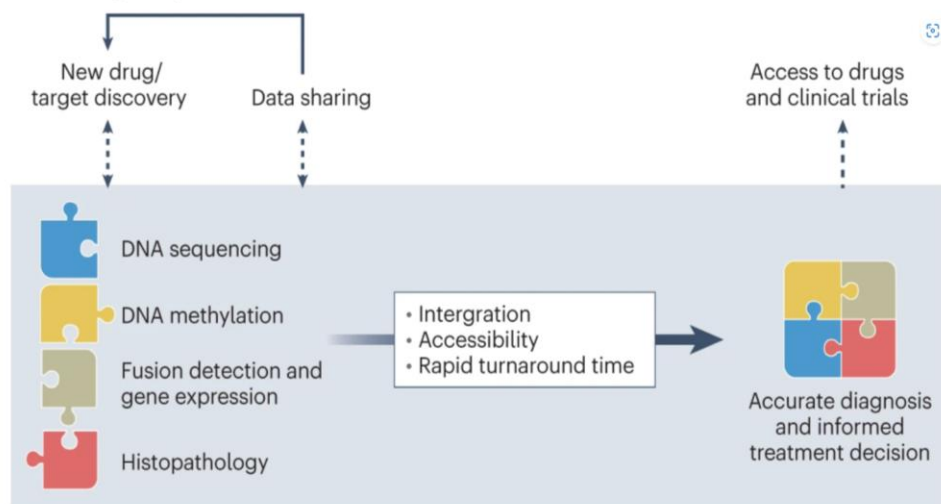
Bandopadhayay & Mardis. Nature Medicine 2023

But even integrated genomics for a diagnostic process has its problems

- Time and cost for multiomic approach
- Cannot replace some single gene tests, e.g. TERT
- Mental laziness – "just send for the NGS and/or methylation" lacking a "pathologic strategy" for reaching a diagnosis

Thank you
Please excuse personal opinions