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Department of Pathology
Division of Molecular Pathology and Diagnostics

Clinical applications of whole genome DNA methylation profiling

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And now one more molecular assay????

We have

- DNA NGS
- RNA NGS
- Expression and targeted assays

Do we really need methylation now?

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BRAF V600E paradox

- Malignant melanoma

(one of the most malignant tumors of the human body)

- Papillary thyroid carcinoma
- Langerhans cell histiocytosis
- Pilocytic Astrocytoma

(one of the most benign tumors of the human body)

Mutations do not tell the full story

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Mutations do not tell the full story

Tumor behavior is highly context (origin/development) dependent

Very different cancers can share the same early driver

If we do better job diagnosing and classifying (brain) tumors and understand their molecular patterns:

-> We can better predict response to therapy

-> We can better predict patient's outcome

-> We can identify high-risk patients

-> Develop novel therapies, design clinical trials

-> We can also develop better early diagnosis tests

(prevention, follow-up)

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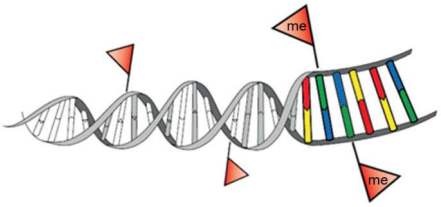
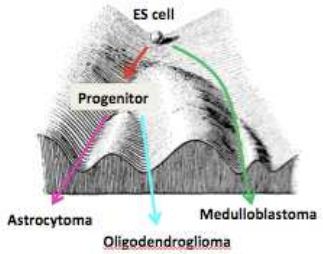
Methylation reflects cell origin

- DNA methylation acts as a “fingerprint” or “memory” that reflects cell lineage and development
- It can reflect particular mutations if those result in distinct changes of methylation/expression patterns
- DNA methylation is a robust biomarker that “survives” after fixation and tissue processing
- DNA methylation is well suited for tumor classification based on cell origin
- Binary nature (methylated/unmethylated) makes it easier for computational analysis





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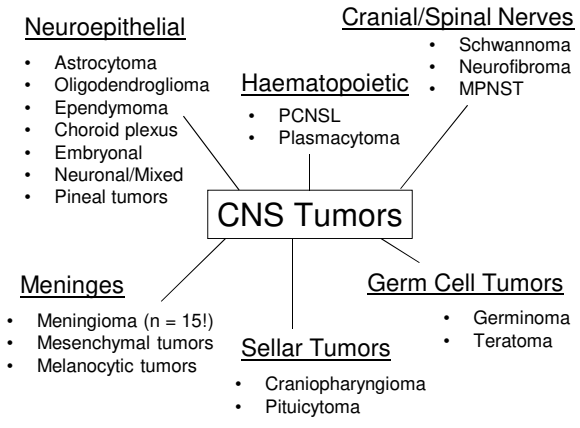
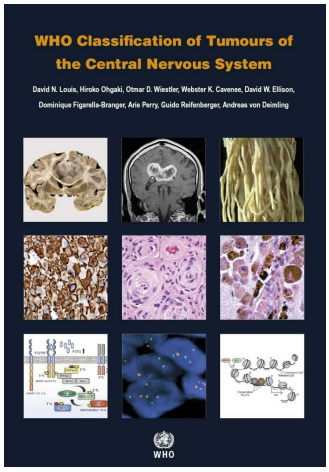
WHO – the gold standard

- Current version (2016), more than 100 different entities,
- Diagnosis is based on histology H&E staining, with some support of molecular features (1p/19q, IDH1/2)





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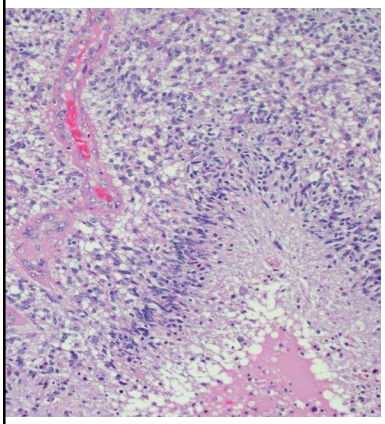
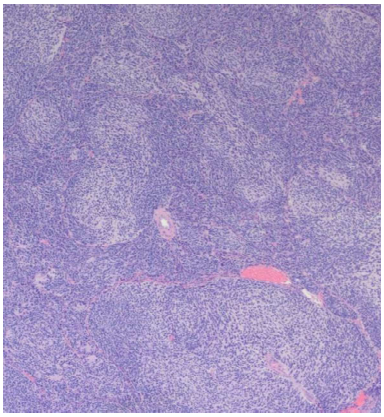
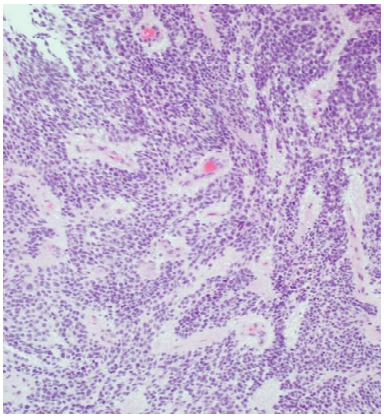
The times they are a-changin'…



GBM
WHO Grade IV



Medulloblastoma,
WHO Grade IV



Ependymoma,
WHO Grade II

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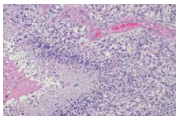
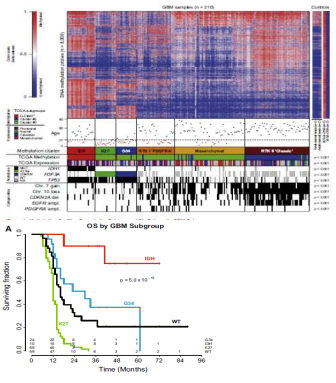
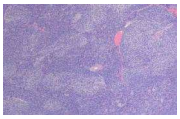
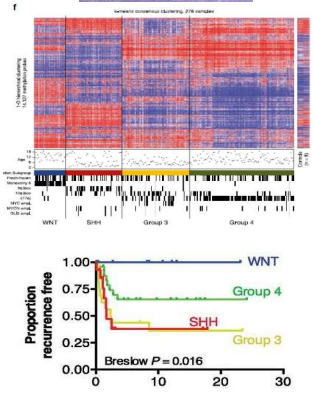
Defining brain tumors molecularly, not histologically



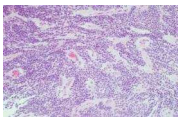


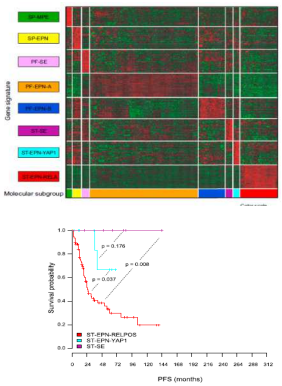
GBM, WHO Grade IV
H3.3 K27 mutated





Medulloblastoma,
WHO Grade IV, Shh subtype





Ependymoma,
WHO Grade II, RELA fusion

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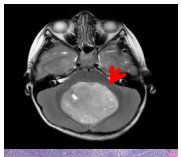
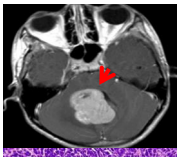
Boy, 4 years old

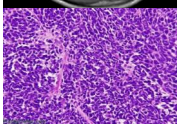
Girl, 5 years old

Two children with a brain tumor





Same clinical diagnosis:
(Medulloblastoma, WHO Grade IV)



Traditional diagnostics

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Boy, 4 years old

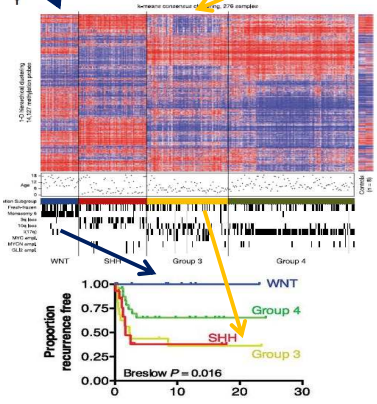
Girl, 5 years old

Two children with a brain tumor

Same clinical diagnosis:
(Medulloblastoma, WHO Grade IV)

Traditional diagnostics

Molecularly different diseases



Precision medicine

Result for two children:
- Different risk of recurrence
- Different long-term survival
- Need for different clinical therapy
- Tailored clinical management
- Targeted therapy

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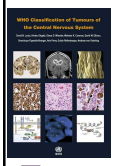
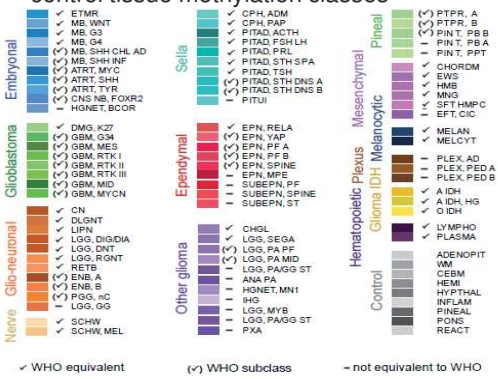
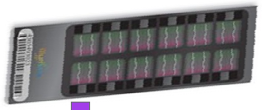
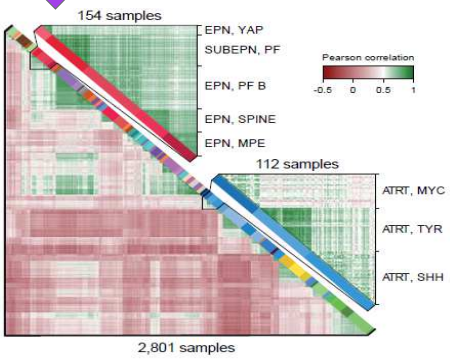
Development of DNA Methylation Diagnostic Classifier



82 CNS tumor methylation classes and 9 control tissue methylation classes







Capper et al, DKFZ, Nature 2018

pairwise Pearson correlation of the 32,000 most variably methylated CpG probes

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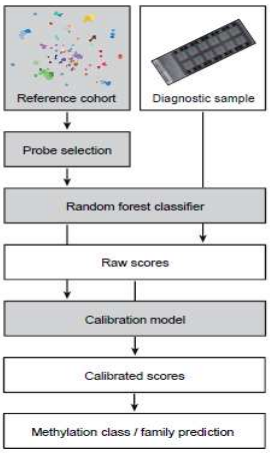
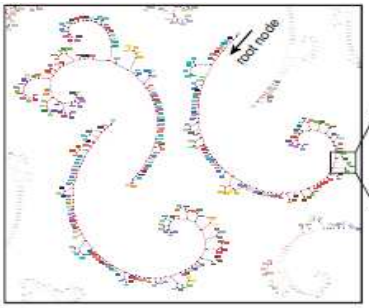
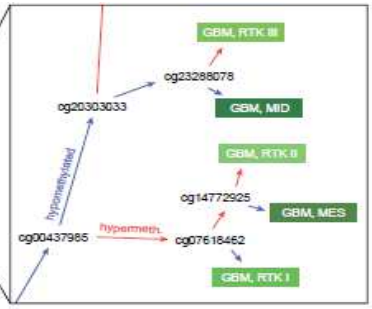
Random Forest Algorithm





3 out of 10,000 decision trees



5 decision points & 5 terminal nodes

Class Prediction:

- A diagnostic sample enters the root node of each of the 10,000 trees in the 'forest'
- At every decision node, the decision path is determined on the methylation level of a single CpG,
- Reaching a terminal node provides the class prediction.

Capper et al, DKFZ, Nature 2018

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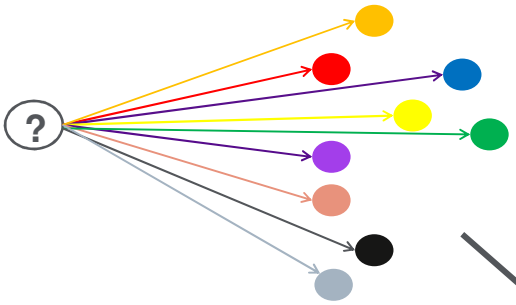
How does it really work…

Tumor of interest → ? → Reference cohort (3500 tumors, 82 entities)



Characterized and orthogonally validated by:

- Morphology (good examples of each entity)
- Sequencing
- Copy number analysis
- Expression profiling

Result:

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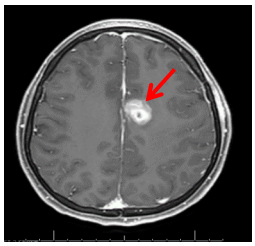
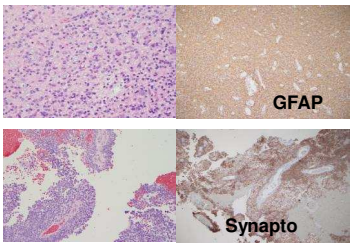
Sample Case #1

17 y.o. F presented with a seizure
- Imaging showed enhancing tumor
- Subtotal resection
- Pathology: “Malignant tumor”





Two expert consultations obtained:

1) Glioblastoma (GBM), WHO Grade IV
   Recommendation: Tumor radiation and glioma (TMZ) chemotherapy

2) Primitive Neuroectodermal Tumor (PNET), WHO Grade IV
   Recommendation: Craniospinal radiation and PNET chemotherapy

Conflicting diagnosis and clinical recommendation
Comes to **NYU** for third opinion

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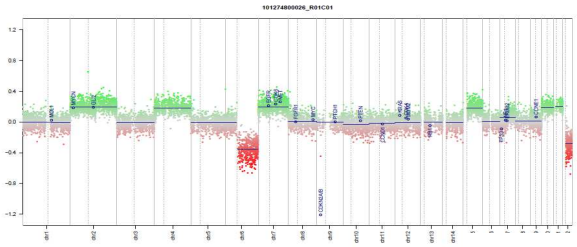
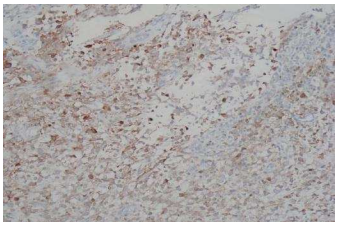
At NYU

- NYU Neuropathology disagrees with both outside diagnoses based on histology
- Performing methylation fingerprinting using 450k methylation array confirms that the tumor clusters with Pleomorphic Xanthoastrocytoma, WHO Grade II
- Further clinical testing identifies BRAF V600E mutation, known genetic driver of PXA

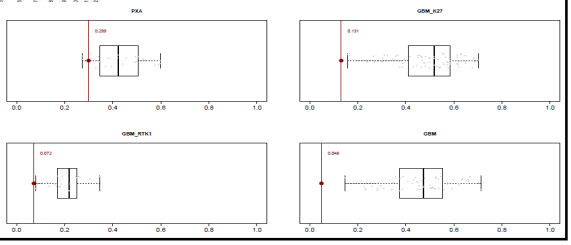
Copy Number Profile





450k Classifier Scores (mnpprediction version 0.1.9)

Score	Abbreviation	Tumor subclass
0.2992	PXA	Pleomorphic xanthoastrocytoma
0.1309	GBM_K27	Glioblastoma H3.3 K27 mut
0.0718	GBM_RTK1	Glioblastoma RTK 1
0.0485	GBM	Glioblastoma



Result from Methylation profiling

- Correct Diagnosis: PXA NOT GBM or PNET
- Correct Grade: WHO Grade II NOT IV
- Correct treatment: Attempt for gross total resection first NOT craniospinal radiation or chemotherapy
- Identified a target for targeted therapy in case of recurrence: vemurafenib showed previously good effect in PXA with BRAF V600E mutation

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Sample Case #2

- 7 y.o. boy presented with progressive headache, nausea and vomiting to Columbia and an MRI demonstrated obstructive hydrocephalus due to a IVth ventricular mass.
- MRI spine showed multiple leptomeningeal metastases.
- Gross total resection
- Diagnosis: Medulloblastoma

- Treated at NYU on CCG 99701 with CSI(54/36 Gy - photons) plus weekly carboplatin and adjuvant chemotherapy and remained in continuous remission

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12 years later….

- At age 19 - incidental finding on surveillance MRI of a new nodular enhancing right sided brachium pontis lesion which grew over a 2 month period.
- Biopsy was performed

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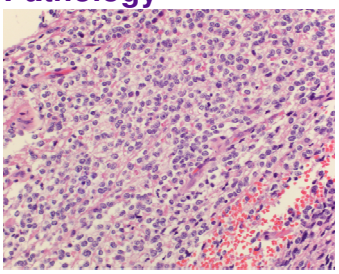
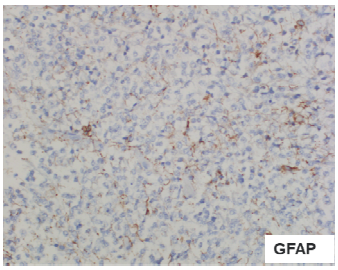
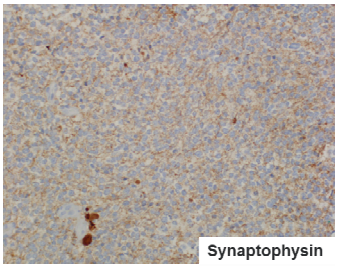
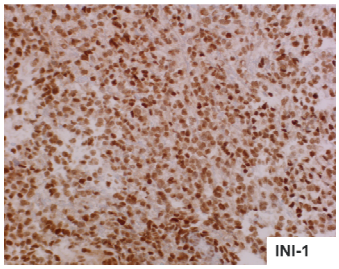
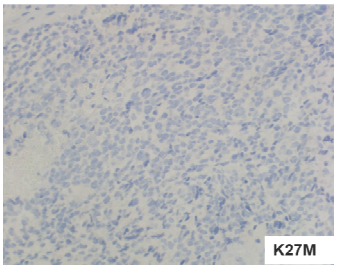
NYU Case 2
Pathology

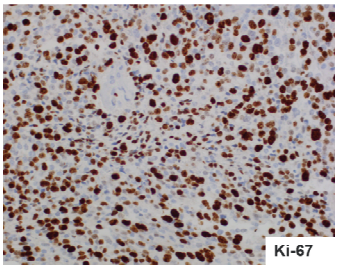












Preliminary histology suggestive of a recurrent medulloblastoma

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Case 2
Pathology: Diagnosis: Glioblastoma WHO Grade IV

Methylation Family Scores

Family Score	Methylation Family
0.87	MTGF_GBM
0.0362	DMG, K27
0.0186	CNS NB, FOXR2
0.0107	MTGF_MB_SHH
0.0086	MTGF_PLEX_T

Methylation Class Scores

Class Score	Methylation Class
0.5988	GBM, MID
0.2501	GBM, RTK I
0.0362	DMG, K27
0.0203	GBM, RTK III
0.0186	CNS NB, FOXR2

Methylation Class Description

The methylation class "glioblastoma, IDH wildtype, subclass midline" is comprised of tumors with a histological diagnosis of glioblastoma, located in midline structures (thalamus, cerebellum, spine). Median age is 13 years (range 2 to 79). Tumors of this class share epigenetic similarities with histone 3 K27M-mutated tumors, but lack this mutation. Mutations of FGFR1 are relatively common, particularly in thalamic tumors. Copy number changes are numerous, the most frequent changes being gain/amplification of PDGFR-alpha and loss of CDKN2A/B (both in over 70% of cases).

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DNA NGS: NYU Langone Genome PACT (Tumor-Normal 607 gene panel)

Molecular Diagnostic Test: NYU Langone GENOME PACT

Results

Next generation sequencing was performed on DNA extracted from normal (Peripheral Blood) and tumor (FFPE) samples.
The following somatic nonsynonymous mutations were identified in the tumor:

Gene / mutation	Function	Site	Coverage	Variant Allele Frequency in the tumor
PDGFRA / p.Asn468Ser	missense variant	exonic	10790	86.70%
SYNE1 / p.Glu2119Gly	missense variant	exonic	488	43.00%
AKAP9 / p.Thr2166Ser	missense variant	exonic	1086	31.80%
MLLT3 / p.Val338SerfsTer66	frameshift variant	exonic	1300	94.80%

The following focal copy number changes were detected in the tumor:

PDGFRA Amplification
CDKN2A Loss
CHIC2 Amplification
FIP1L1 Amplification
PSIP1 Amplification

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Result from Methylation profiling

- Correct Diagnosis: GBM NOT Recurrent Medulloblastoma
- Correct treatment: GBM

- Epidemiological questions: what fraction of “recurrent medulloblastomas” areactually secondary GBMs….?
- Survival analysis and assessment of therapeutic efficacy
- Important for design of future clinical trials
- De-escalation of primary tumor treatment to decrease the risk of secondary tumors

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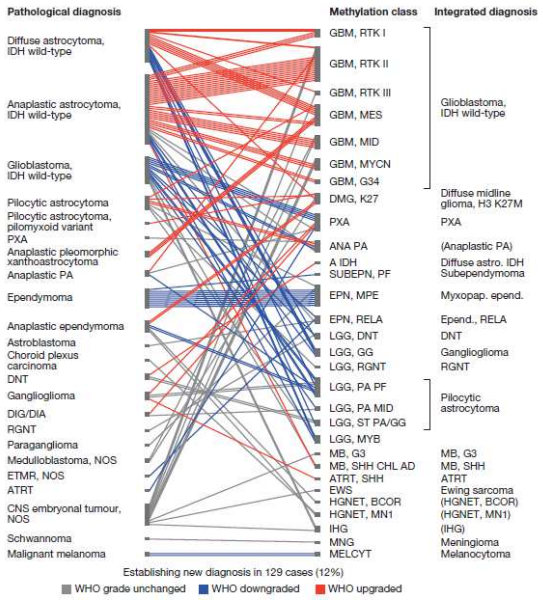
Impact of methylation profiling on diagnostic accuracy

12-14% of brain tumors may be misdiagnosed using current routine diagnostic tools



Capper et al, DKFZ, Nature 2018

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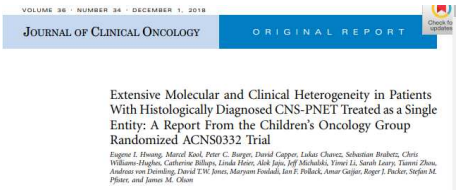
Misdiagnosis has a significant impact on clinical trials

ACNS0332 trial

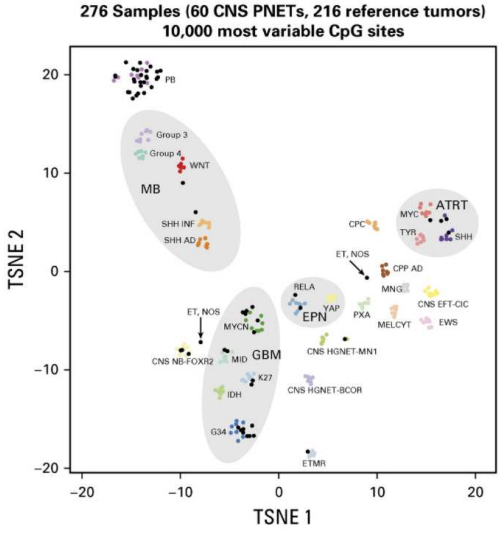
- Phase III, four-arm prospective trial (COG)
- Children age 3-22 years with newly diagnosed CNS-PNET / Pineoblastoma
- Randomly assigned (1:1) to receive carboplatin during radiation and/or adjuvant isotretinoin after standard intensive therapy
- Enrollment of patients was discontinued (fail)
- Molecular profiling later revealed tumor heterogeneity that was not detectable at protocol inception.
- **71% of "PNET cases"** represented molecularly defined entities that <u>were not intended for trial inclusion</u>


VOLUME 36 · NUMBER 34 · DECEMBER 1, 2018

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Extensive Molecular and Clinical Heterogeneity in Patients With Histologically Diagnosed CNS-PNET Treated as a Single Entity: A Report From the Children's Oncology Group Randomized ACNS0332 Trial

Eugene I. Hwang, Marcel Kool, Peter C. Burger, David Capper, Lukas Chavez, Sebastian Brabetz, Chris Williams-Hughes, Catherine Billups, Linda Heier, Alok Jaju, Jeff Michalski, Yimei Li, Sarah Leary, Tianni Zhou, Andreas von Deimling, David T.W. Jones, Maryam Fouladi, Ian F. Pollack, Amar Gajjar, Roger J. Packer, Stefan M. Pfister, and James M. Olson



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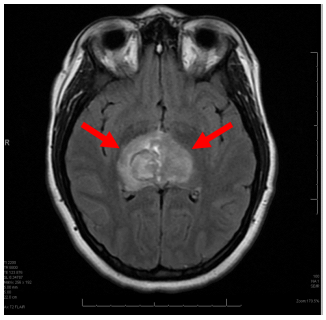
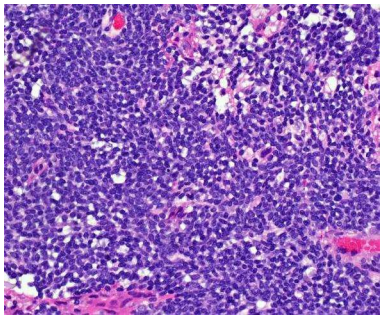
Combining a power of methylation classifiers: building epigenetic maps of cancer





22 y.o. F
- Diagnosis of pineoblastoma 1 year ago
- Status post CSI and adjuvant chemotherapy per high risk COG medulloblastoma protocol
- Recurrent disease with local recurrence diagnosis

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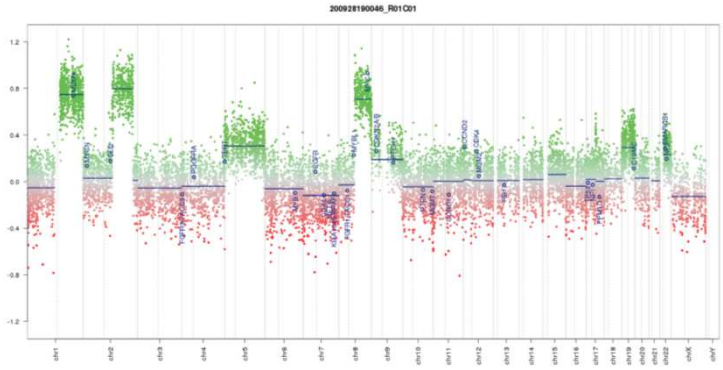
At NYU

- No match by CNS brain tumor classifier

Copy number variation profile



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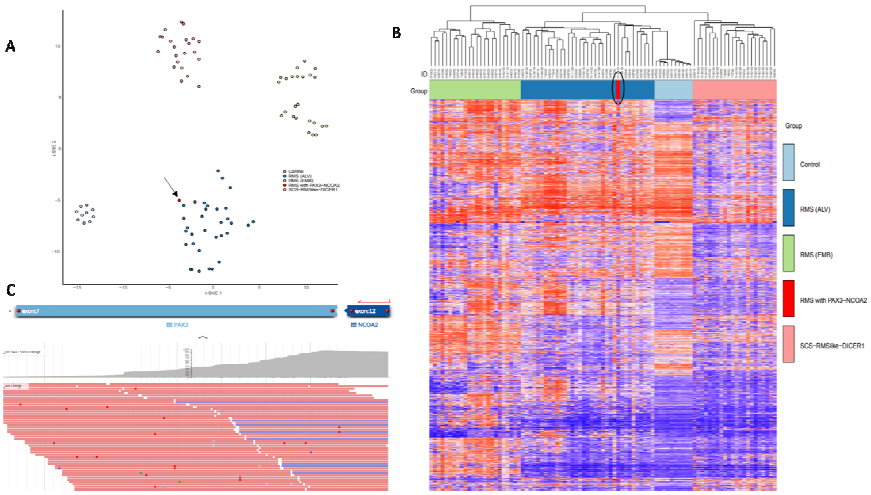
Brain tumor clusters with alveolar rhabdomyosarcoma



Jour et al, J of Neuropathology and Experimental Neurology, 2019

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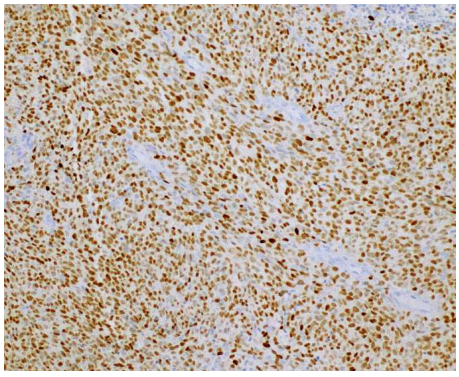
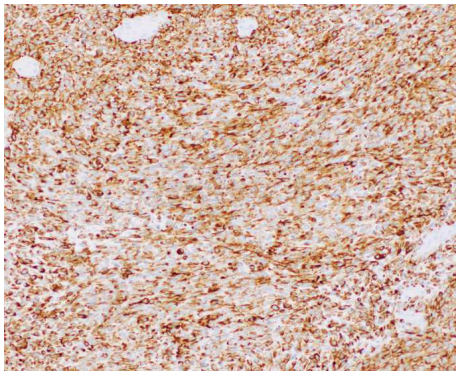
Additional IHC work performed



Myogenin



Desmin

Diagnosis: Rhabdomyosarcoma, with PAX3-NCOA2 fusion

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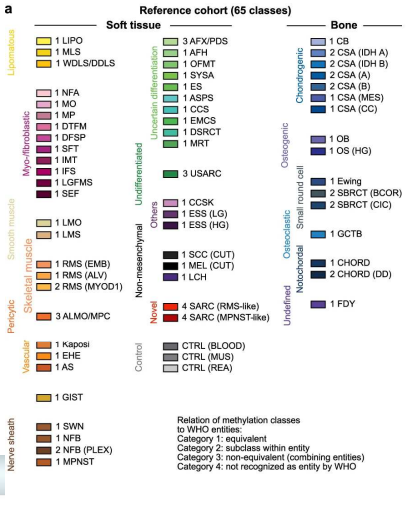
Sarcoma classifier



nature COMMUNICATIONS

ARTICLE

OPEN

Sarcoma classification by DNA methylation profiling

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-20603-4 ARTICLE

Christian Koelsche[1,2,3,90], Daniel Schrimpf[1,2,90], Damian Stichel[2,90], Martin Sill[4,5,90], Felix Sahm[1,2],

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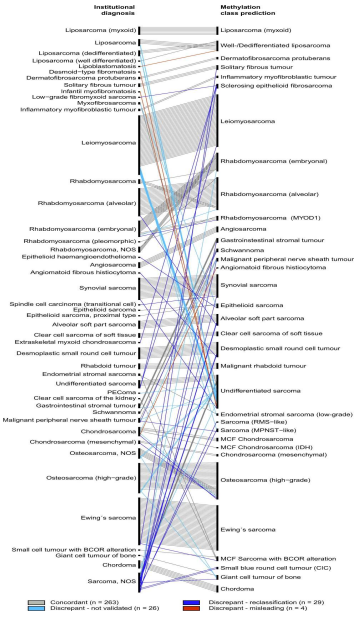
Identifying diagnostic discrepancies



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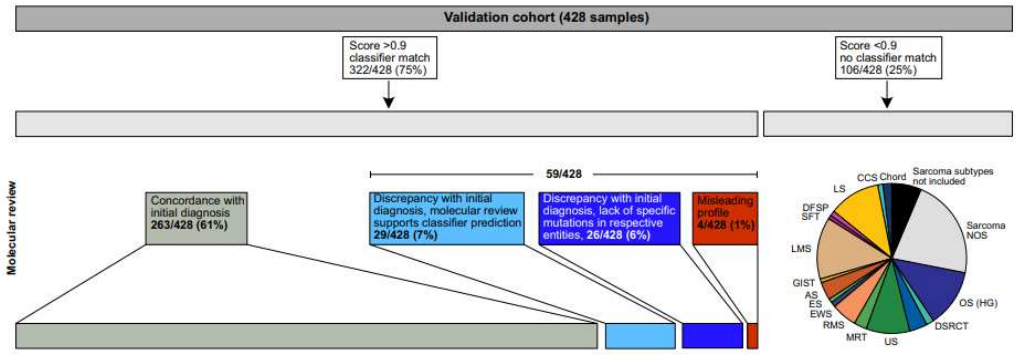
Identifying diagnostic sarcoma discrepancies



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Solving a mystery of a "cancer of unknown origin"

- 65 y.o. male with new onset seizures
- Imaging shows hemorrhagic mass in the L parietal lobe
- Histology: poorly differentiated neoplasm ("negative for everything except vimentin")
- Whole body CT scan negative for any primary tumor
- Conclusion: high grade glioma

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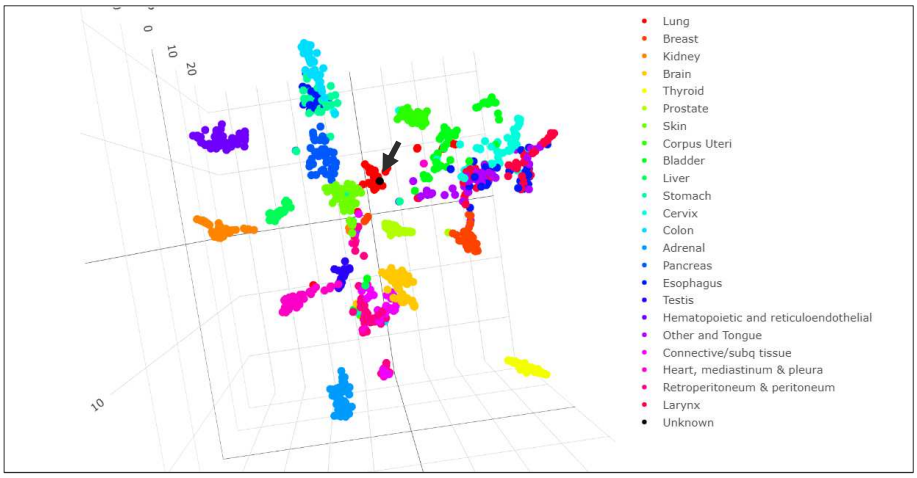
DNA methylation profiling: comparing with ~8000 samples from ~50 cancer entities: Lung NSC



Patient was rescanned, a small lung nodule identified, biopsy showed poorly differentiated carcinoma similar to the brain metastasis sample

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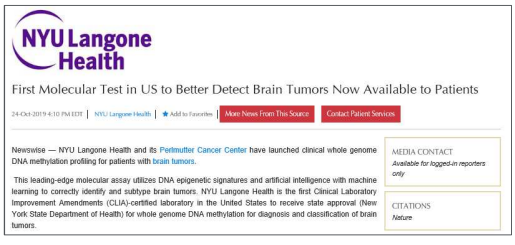
Current applications at NYU

- Whole genome 850k methylation array profiling is currently applied at NYU in diagnostics for:
  - All pediatric and adult brain tumors diagnosed at NYU
  - Cases in consultation with unusual presentation, unclear histology, unusual clinical course
  - Pediatric sarcoma, unusual malignant tumors of childhood
  - Tumors of unknown origin (in development),& Gyn, ENT, Breast, GU

In 2019 NYU Langone Molecular Pathology received NYS DOH approval and started offering methylation profiling as the first CLIA and NYU State approved clinical test for brain tumor diagnostics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First Molecular Test in US to Better Detect Brain Tumors Now Available to Patients

24-Oct-2019 4:10 PM EDT | NYU Langone Health | ★ Add to Favorites | More News From This Source | Contact Patient Services

Newswise — NYU Langone Health and its Perlmutter Cancer Center have launched clinical whole genome DNA methylation profiling for patients with brain tumors.

This leading-edge molecular assay utilizes DNA epigenetic signatures and artificial intelligence with machine learning to correctly identify and subtype brain tumors. NYU Langone Health is the first Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory in the United States to receive state approval (New York State Department of Health) for whole genome DNA methylation for diagnosis and classification of brain tumors.

MEDIA CONTACT
Available for logged-in reporters only

CITATIONS
Nature

https://www.newswise.com/articles/first-molecular-test-in-us-to-better-detect-brain-tumors-now-available-to-patients

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Have you joined the methylation hub???

NYU DNA Methylation hub:

- open access
- collaborators can send clinical or research cases for profiling
- pay only cost of the reagents
- get our pipeline results
- get raw data to integrate with their in-house analysis (WES, transcriptome)
- have access to data from other cohorts
- sharing unclassifiable cases to establish new tumor entities

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Conclusion

- We are entering molecular pathology era of precision diagnostics and in-depth analysis that will allow us more accurately stratify (brain) tumor patients for clinical management.
- Integration of genomic, transriptomic and epigenetic data requires data & resource sharing to collect sufficient evidence to change standard of care
- DNA methylation is a critical diagnostic and prognostic biomarker for diagnosis and classification of brain tumors
- AI and machine learning can transform molecular diagnostics but close interaction between molecular and surgical pathology is critical

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Thank you

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