

Molecular classification and characterization of pediatric brain tumors –

need for more and better preclinical models

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Edinburgh, February 27th, 2020



KiTZ

Hopp Children's Cancer Center
at the NCT Heidelberg

dkfz.

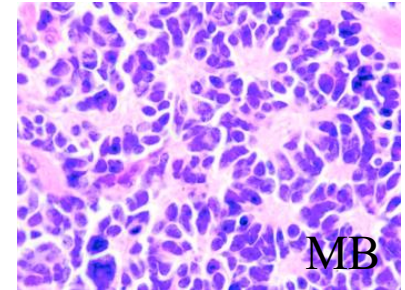
GERMAN
CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION



50 Years – Research for
A Life Without Cancer

WHO Classification CNS tumors

- Updated version 2016: more than 100 different entities
- Mostly (still) histologically defined entities



Neuroepithelial

- Astrocytoma
- Oligodendroglioma
- Ependymoma
- Choroid plexus
- Embryonal
- Neuronal/Mixed
- Pineal tumors

Cranial/Spinal Nerves

- Schwannoma
- Neurofibroma
- MPNST

Haematopoietic

- PCNSL
- Plasmacytoma

CNS Tumors

Meninges

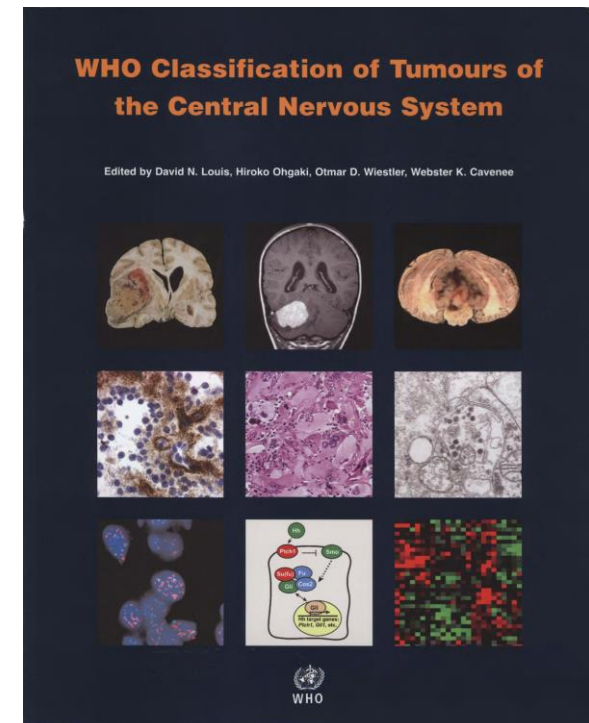
- Meningioma (n = 15!)
- Mesenchymal tumors
- Melanocytic tumors

Germ Cell Tumors

- Germinoma
- Teratoma

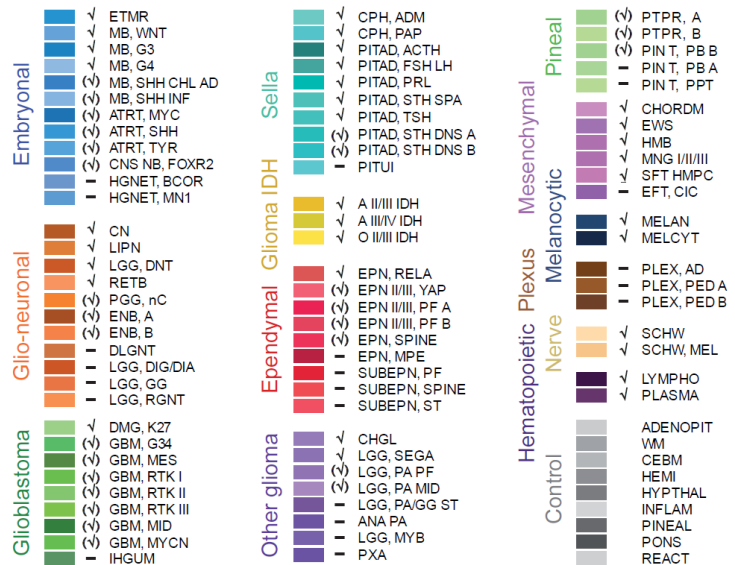
Sellar Tumors

- Craniopharyngioma
- Pituicytoma



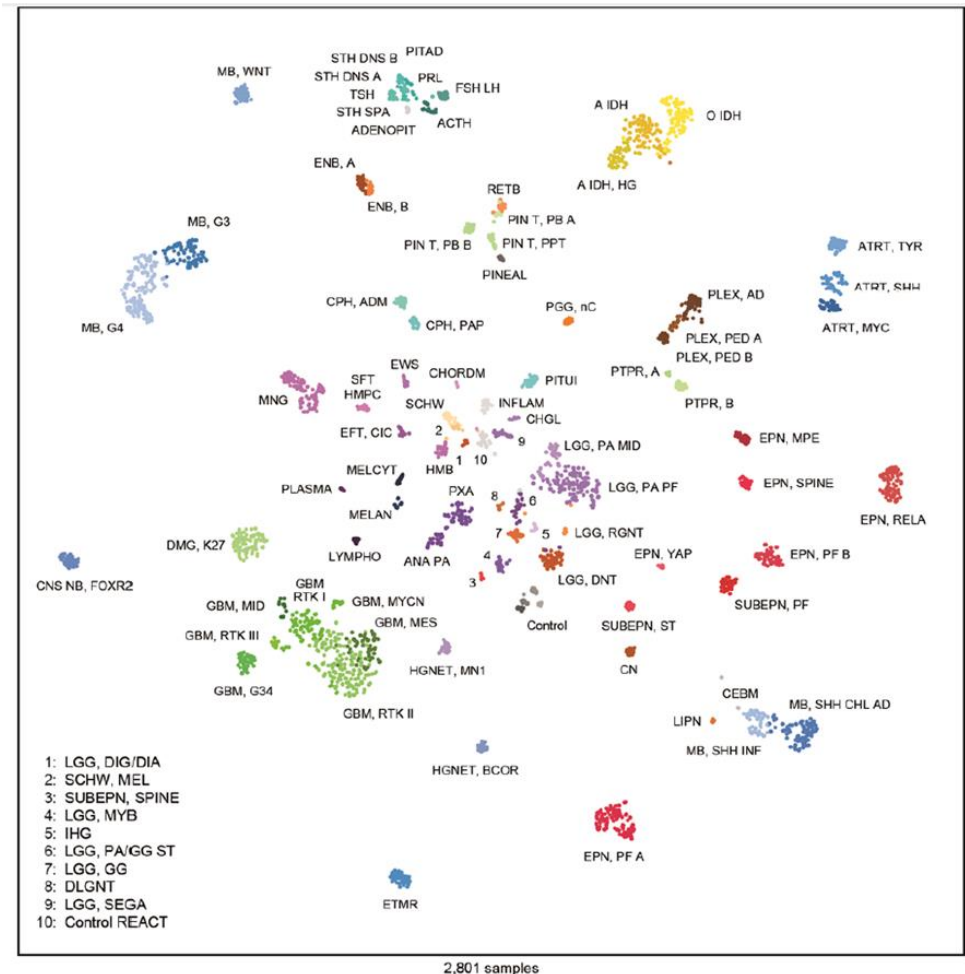
Using DNA methylation for classification purposes

Development of a large, high-quality tumor reference set with good annotation



Identification of at least 82 distinct molecular subtypes of CNS tumors

Update will contain >170 distinct subtypes!



Capper, Jones, Sill, Hovestadt et al., Nature 2018

medulloblastoma



medulloblastoma



medulloblastoma

medulloblastoma



medulloblastoma

medulloblastoma

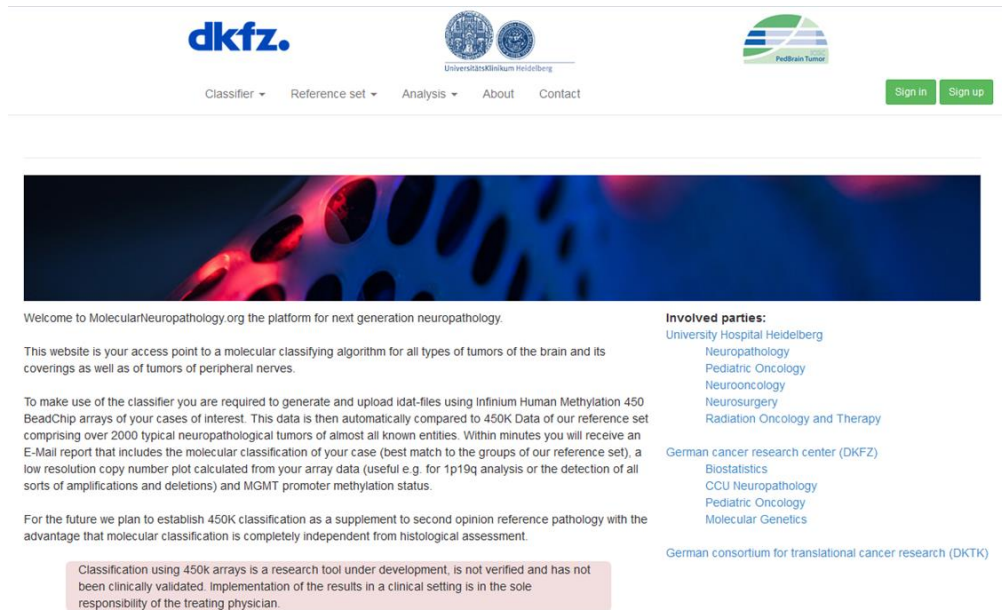


medulloblastoma

medulloblastoma

Methylation classifier website

- Classifier tool and information on molecular classes freely available at: www.molecularneuropathology.org



The screenshot shows the homepage of the MolecularNeuropathology.org website. At the top, there are logos for dkfz., Universitätsklinikum Heidelberg, and Pediatric Tumor. Below the logos is a navigation bar with links: Classifier, Reference set, Analysis, About, and Contact. There are also 'Sign in' and 'Sign up' buttons. A large banner image shows a microscopic view of brain tissue. Below the banner, the text reads: 'Welcome to MolecularNeuropathology.org the platform for next generation neuropathology.' It then describes the website as an access point to a molecular classifying algorithm for all types of tumors of the brain and its coverings as well as of tumors of peripheral nerves. It explains that to use the classifier, users need to generate and upload idat-files using Infinium Human Methylation 450 BeadChip arrays. It mentions that the data is compared to a reference set of over 2000 typical neuropathological tumors. Within minutes, users will receive an E-Mail report including the molecular classification of their case (best match to the groups of our reference set), a low resolution copy number plot calculated from their array data (useful e.g. for 1p19q analysis or the detection of all sorts of amplifications and deletions), and MGMT promoter methylation status. It also states that for the future, they plan to establish 450K classification as a supplement to second opinion reference pathology with the advantage that molecular classification is completely independent from histological assessment. A note at the bottom states: 'Classification using 450K arrays is a research tool under development, is not verified and has not been clinically validated. Implementation of the results in a clinical setting is in the sole responsibility of the treating physician.'

Involved parties:

- University Hospital Heidelberg
 - Neuropathology
 - Pediatric Oncology
 - Neurooncology
 - Neurosurgery
 - Radiation Oncology and Therapy
- German cancer research center (DKFZ)
 - Biostatistics
 - CCU Neuropathology
 - Pediatric Oncology
 - Molecular Genetics
- German consortium for translational cancer research (DKTK)

Already >37000 external profiles analyzed!



The screenshot shows the 'Methylation profiling report' page. It includes logos for dkfz., GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION, Heidelberg University Hospital, and MolecularNeuropathology.org. The report is for 'Sample 2' with Satrix ID '9444375150_R01C01'. The material type is 'FFPE DNA', the gender is 'female', and the supplier diagnosis is 'Medulloblastoma (WHO grade IV)'. The automatic prediction is 'medulloblastoma, WNT' with a calibrated score of 0.99 and an interpretation of 'match'. A legend indicates that a green checkmark means 'Match (score >= 0.9)', a red X means 'No match (score < 0.9): possibly still relevant for low tumor content and low DNA quality cases', and a green dot means 'Match to MC family member (score >= 0.5)'.

Methylation profiling report

Supplier information

Sample identifier:	Sample 2	Automatic prediction
Satrix ID:	9444375150_R01C01	Array type: 450k
Material type:	FFPE DNA	Material type: FFPE DNA
Gender:	female	Gender: female
Supplier diagnosis:	Medulloblastoma (WHO grade IV)	Legend: ✓ OK ✗ Supplier information or prediction not available ✗ Warning, mismatch of prediction and supplier information

Brain tumor methylation classifier results (v11b2)

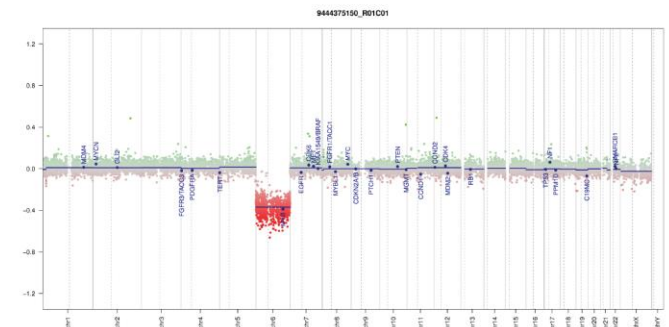
Methylation classes (MCs with score >= 0.3)	Calibrated score	Interpretation
methylation class medulloblastoma, WNT	0.99	match

Legend: ✓ Match (score >= 0.9) ✗ No match (score < 0.9): possibly still relevant for low tumor content and low DNA quality cases. ● Match to MC family member (score >= 0.5)

Class descriptions

Methylation class medulloblastoma, WNT: The methylation class "medulloblastoma, WNT" is comprised of tumors diagnosed as Medulloblastoma, genetically defined, WNT-activated. Histologically most cases fall into the classic variant, sometimes into the large cell/anaplastic variant. Tumors are located in the cerebellum, typically in the vermis. Median age is 12 years (range 6 to 27). Monosomy of chromosome 6 (>80%) together with mutation of CTNGB1 is (almost) pathognomonic for this class.

Copy number variation profile

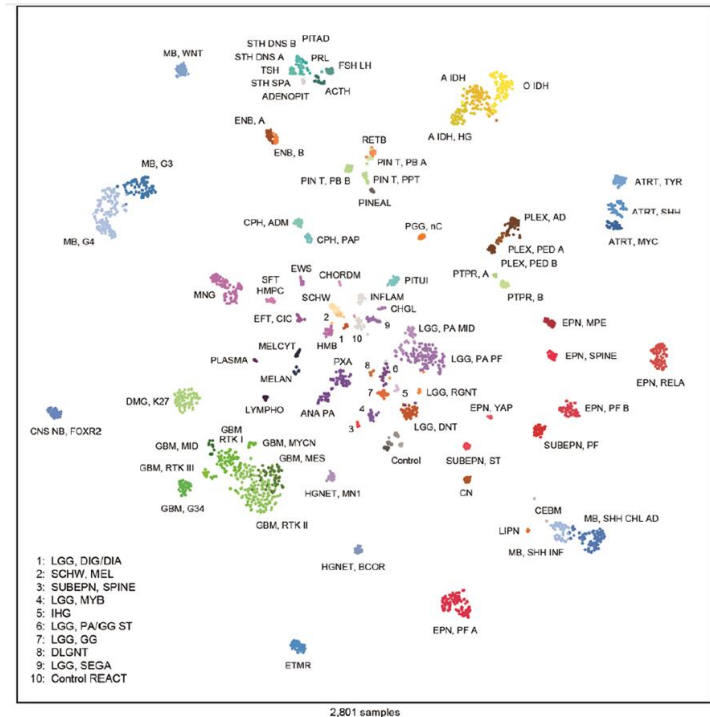


Depiction of chromosome 1 to 22 and (X,Y) if automatic prediction was successful. Gains/amplifications represent positive, losses negative deviations from the baseline. 29 brain tumor relevant gene regions are highlighted for easier assessment. (see Hovestadt & Zapatka, <http://www.bioconductor.org/packages/develop/bioc/html/consumee.html>)

Many distinct molecular entities of brain tumors require multiple distinct model systems for preclinical studies

Model systems:

- Cell lines
- Organoids
- GEMMs
- In utero electroporation mouse models
- PDOX models



Patient Derived Orthotopic Xenograft (PDOX) models

Aim is to generate a **large repertoire of PDOX models** reflecting the many different **molecular subtypes** of pediatric brain cancer, which can be shared in the pediatric cancer community for preclinical studies

- **Generate** PDOX Models
- **Characterize** established models (and correlate them to patient subgroups)
- **Preclinical studies**
- **Understand mechanisms of therapy resistance**

Collaborative Approach between different labs:

- Seattle (James Olson, Sarah Leary)
- San Diego (Robert Wechsler-Reya, Jessica Rusert))
- Houston (Xiao-Nan Li)
- Heidelberg (Till Milde)
- Paris (Olivier Ayrault)
- Vienna (Walter Berger, Johannes Gojo)

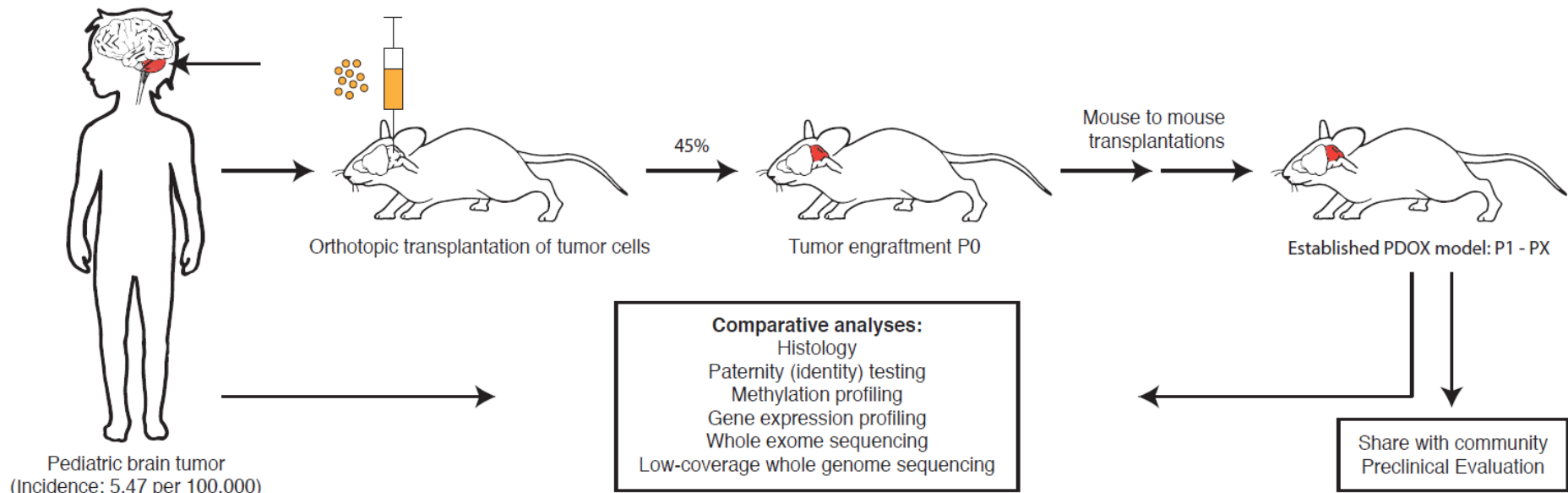


Sebastian Brabetz



Norman Mack

Generation and characterization of PDOX models



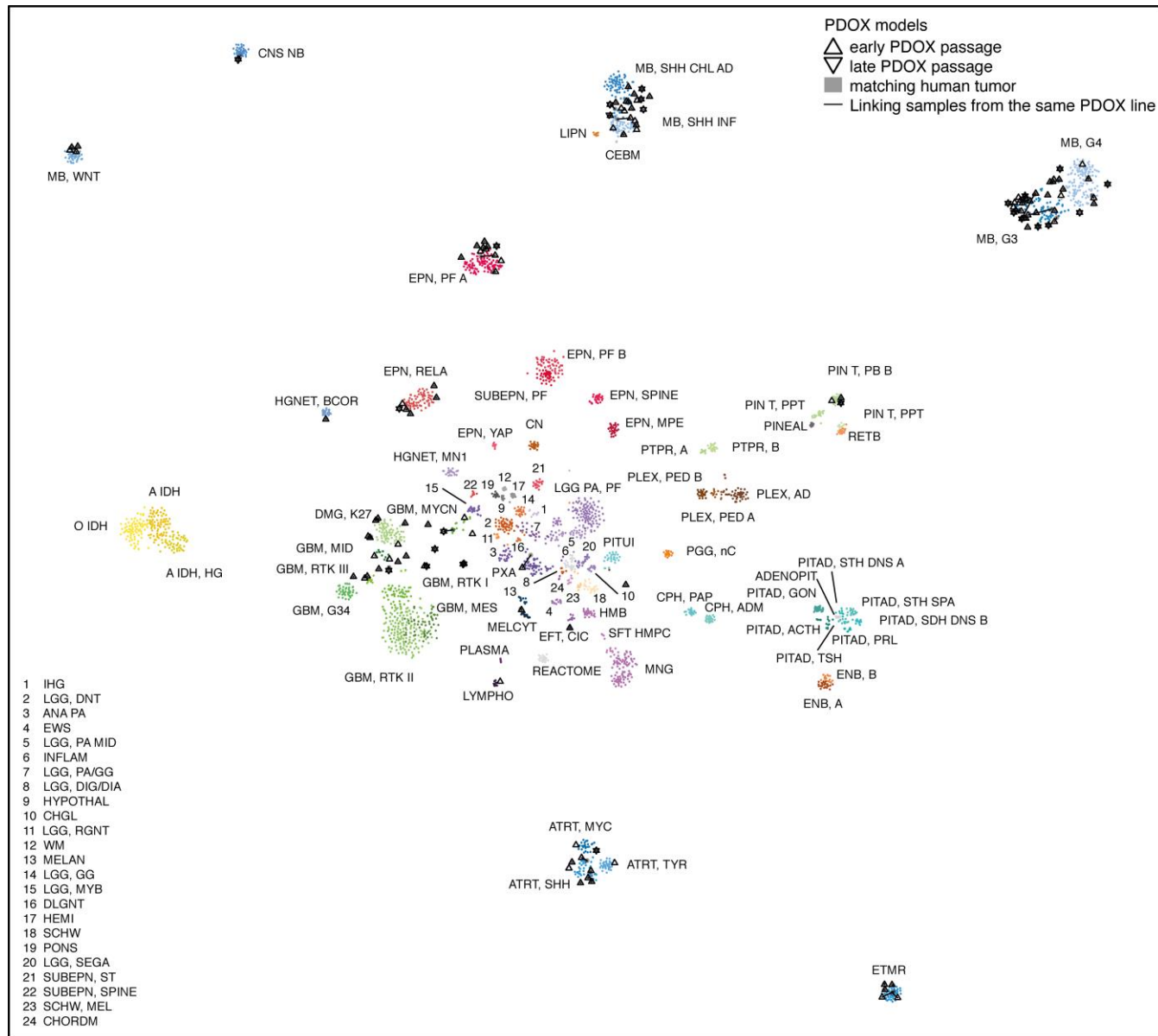
Brabetz et al., Nature Medicine 2018

Molecular Characterization (PDOX, primary tumor, blood):

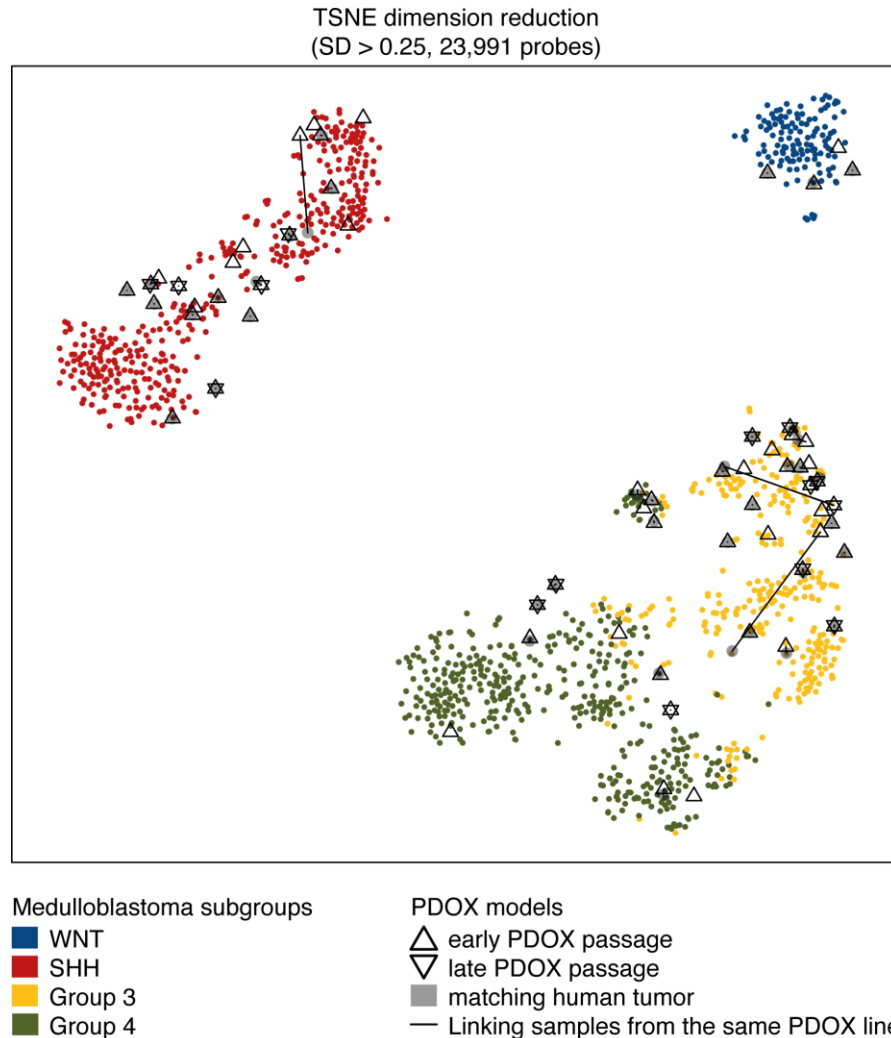
- 450K Methylation Profiling: Tumor Classification, CNVs
- Gene Expression Profiling: pathways, drug targets
- Whole Exome Sequencing: SNVs / indels
- Low coverage WGS: SVs, CNVs

**Currently >130 PDOX models
fully analyzed**

Molecular classification of 130 brain tumor PDOX



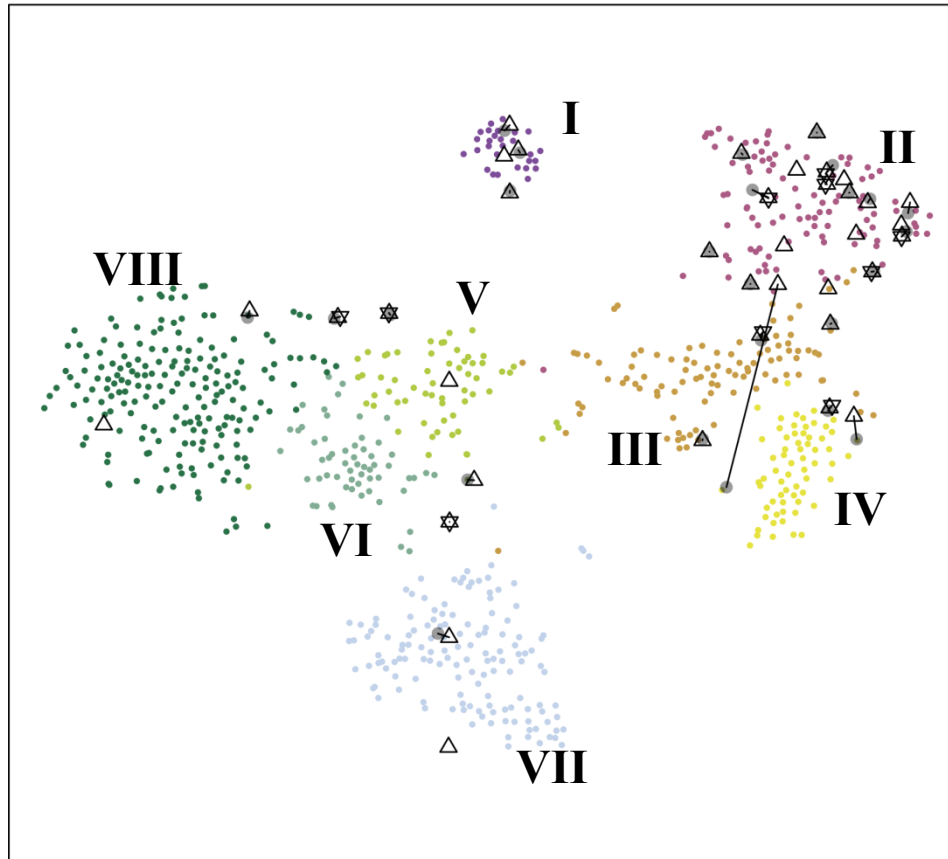
Molecular classification of medulloblastoma PDOX (n = 62)



MB WNT:	4
MB SHH:	21
MB GRP3:	24
MB GRP4:	13

PDOX can model the heterogeneity of Group 3 and Group 4 medulloblastoma

TSNE dimension reduction
(SD > 0.25, 14,759 probes)

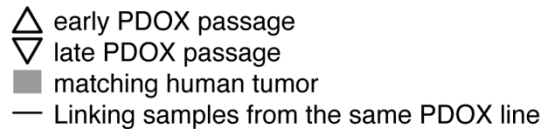


I:	4
II:	19 (MYC)
III:	3
IV:	2
V:	3
VI:	2
VII:	2
VIII:	2

Group 3/4 subgroups

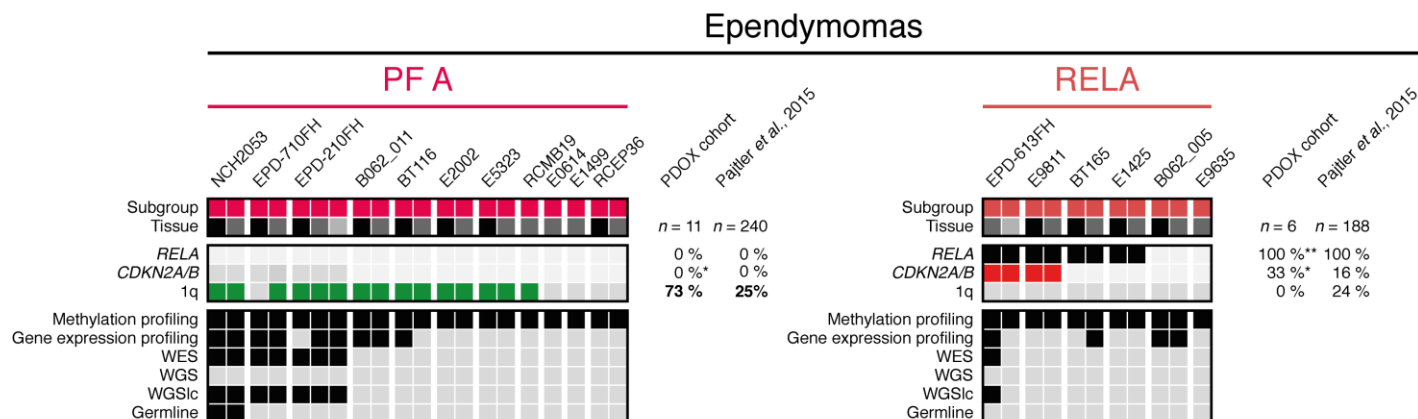


PDOX models

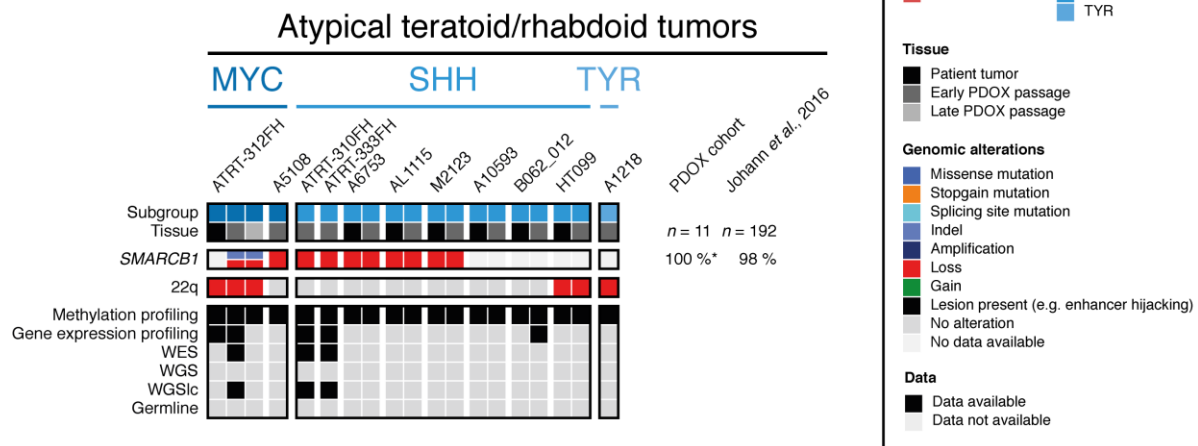


Bias for most aggressive tumors even within tumor subgroups

a



b



PDX data sheets and PDX Explorer: Making data available

How to make the data accessible?

How information is usually shared:

Figures in paper + supplementary material

Raw data: EGA + GEO

Disadvantages:

Data is split across multiple figures and excel sheets

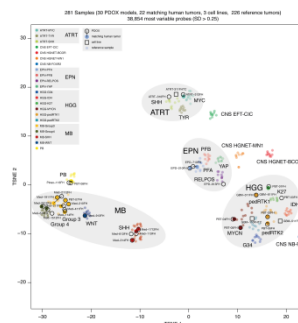
Raw data is not very useful to a broad audience

Our approach for a solution:

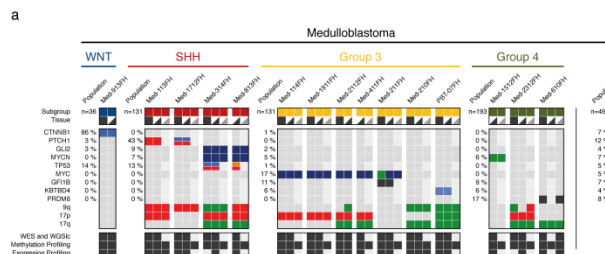
PDX data sheets

PDX Explorer in R2 (r2.amc.nl)

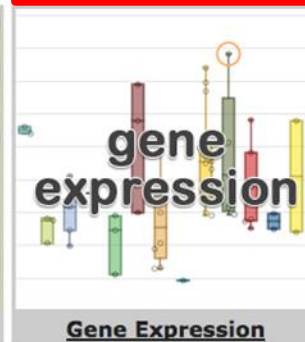
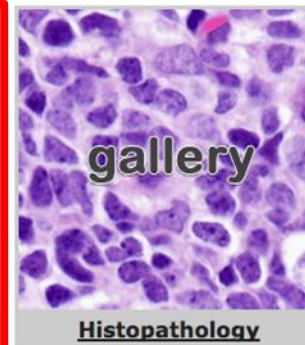
Easy ordering of lines via www.btrl.org



S1-S23



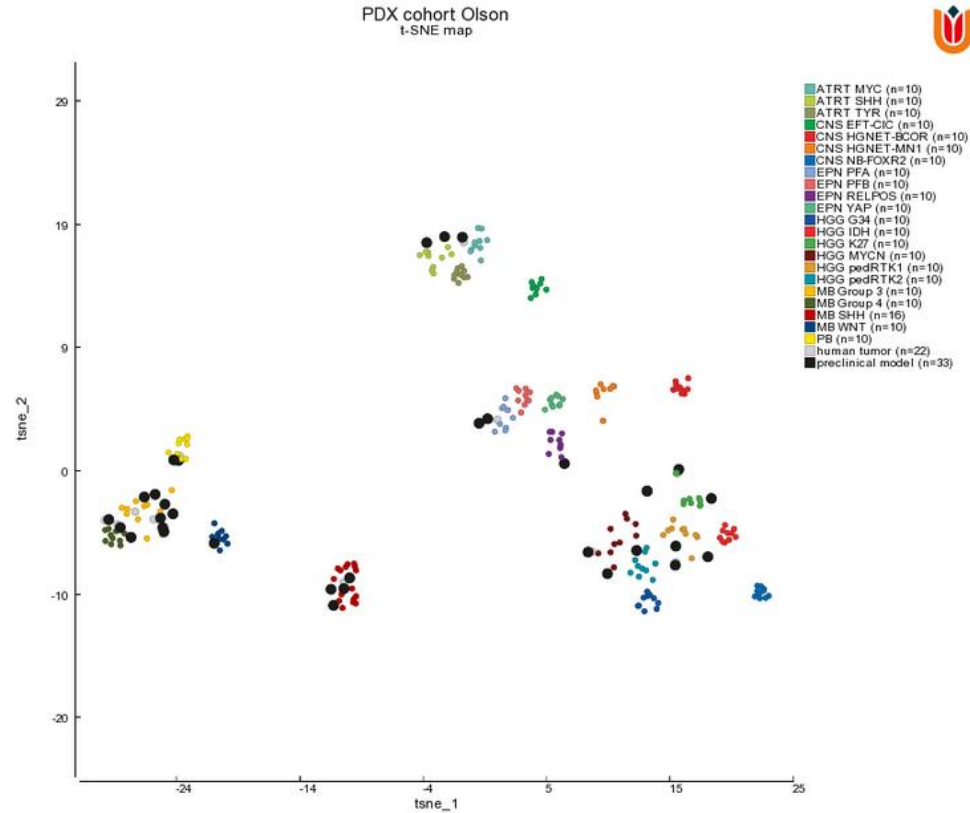
PDX Explorer (r2.amc.nl)



96	84199298	-	GT	G	exonic	RAS99
	4662660		A	A	exonic	RAS97
76	4837677	rs146098580	C	T	exonic	ERAS
512	192973513	rs150318874	G	A	exonic	HRA5L
	42836648		G	A	exonic	RASGF1
48	7992149	rs141767871	G	A	exonic	RASGF1
61	2577892	rs121913527	C	T	exonic	KRAS
83	25780		C	T	exonic	KRAS
83	257892	rs121913527	C	T	exonic	KRAS
	141571544		T	A	exonic	RASA2
39	1556		C	T	exonic	RASA3L
657	20675461		CA	C	exonic	RAS95
	82066727		C	T	exonic	RASGF1
48	25930285	rs121913530	C	G	exonic	KRAS
743	115258744	rs121454366	C	G	exonic	NRAS
746	115258747	rs121913237	C	T	exonic	NRAS
747	115258748	rs121913250	C	T	exonic	NRAS

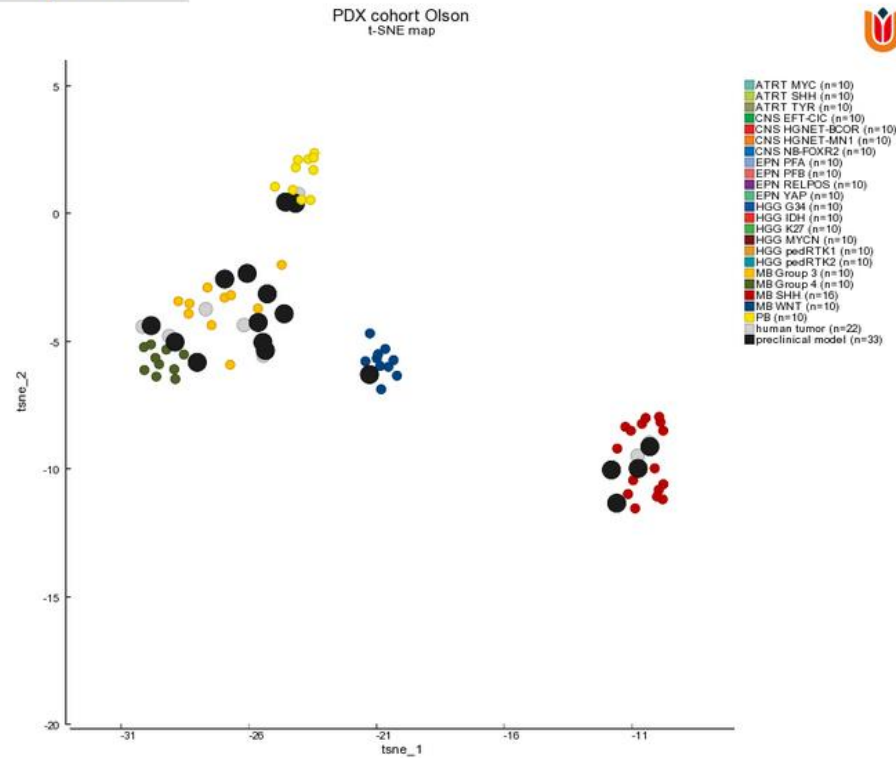
[illegible]

PDX Explorer



[Directly Explore Molecular Data](#)

Explorer



MB

mb_grp3

med-114fh

med-1911fh

med-210fh

med-2112fh

med-211fh

med-411fh

pbt-07fh

mb_grp4

med-1512fh

med-2312fh

med-610fh

mb_shh

med-113fh

med-1712fh

med-314fh

med-813fh

mb_wnt

med-913fh

Fact sheet

Download PDF →

Patient information

Model information

Molecular data

In depth molecular data →

Explorer

MB

PDF

Adobe

med-211fh

Alternative names:

1.patient

age: 2.8

gender: male

location: cerebellum

diagnosis: medulloblastoma, classic

pre-treatment: none

source: surgery

stage: m0

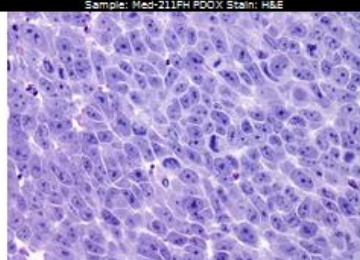
treatment_follow_up:

efs_(months): > 60

os_(months): > 60

consent:

Sample: Med-211FH PDX Stain: H&E



Previous Next

Explore Histology

pathology_of_human_tumor: h&e stained sections show a heterogeneous "small round blue cell" neoplasm. In many areas the cells have dense, hyperchromatic, irregularly shaped nuclei with scant amounts of light pink cytoplasm. there is moderate mitotic activity. focally, the neoplasm is growing in sheets and small nests. much of the tumor contains a rich neuropil background and ganglion cell differentiation is easily identified occasionally binucleated ganglion cells are noted. only very focal frank nodule formation is apparent. Immunoperoxidase staining for In1-1 shows diffuse nuclear expression. some areas of the tumor have marked neuronal differentiation with easily identified ganglion cells. neuropil is moderately abundant.

2.model

mouse_strain: nod acid gamma (nsg)

site_of_transplantation: cerebellum

protocol: olson lab pdx protocol

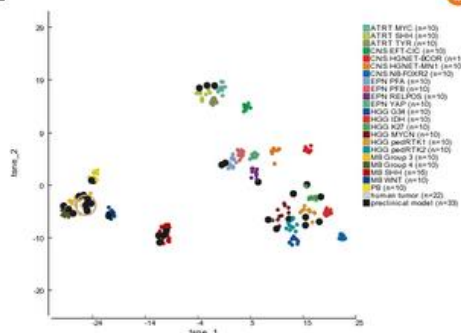
days_to_p0_p1_p2: 64 49 42

pi: james m. olson

contact: <http://www.btrf.org/product/med-211fh/>

PDX cohort Olson

t-SNE map



3.molecular

entity: medulloblastoma

subgroup: mb grp3

curated_lesions: myc amplification, gfi1b activation (structural rearrangement + overexpression)

hg19:

GeneBrowser



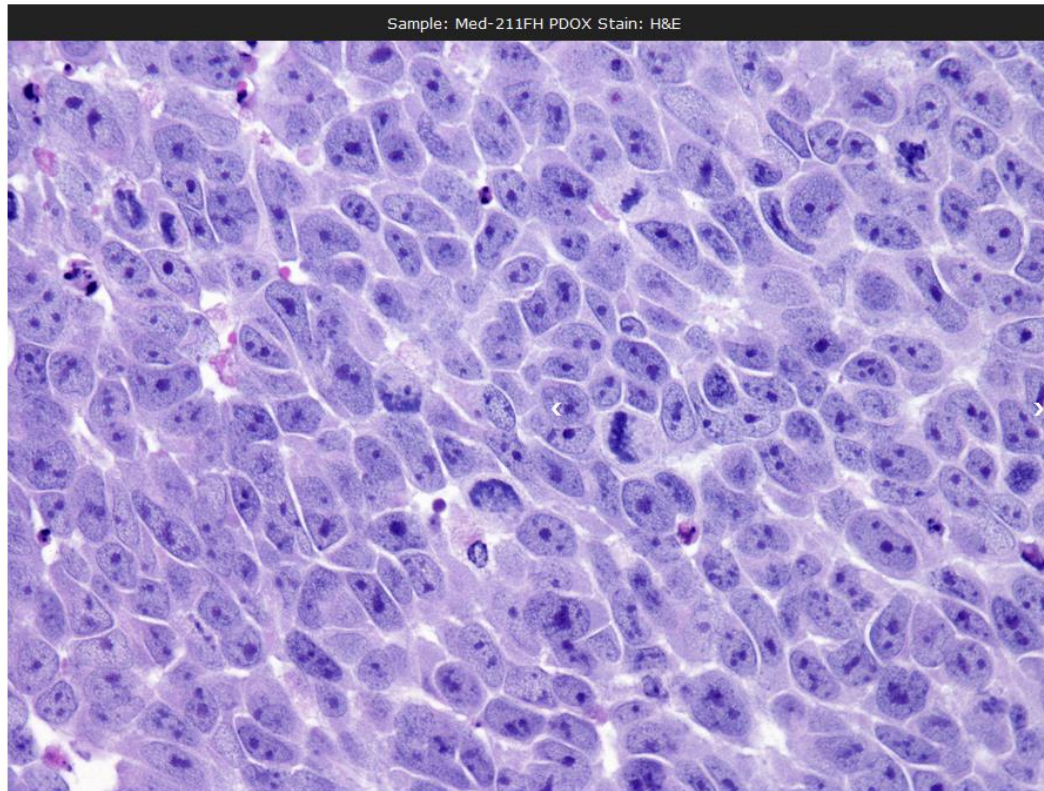
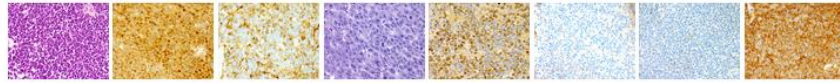
View karyo in GenomeBrowser

plot: med-211fh_p2

Explore More Molecular Data

← Histology and IHC gallery

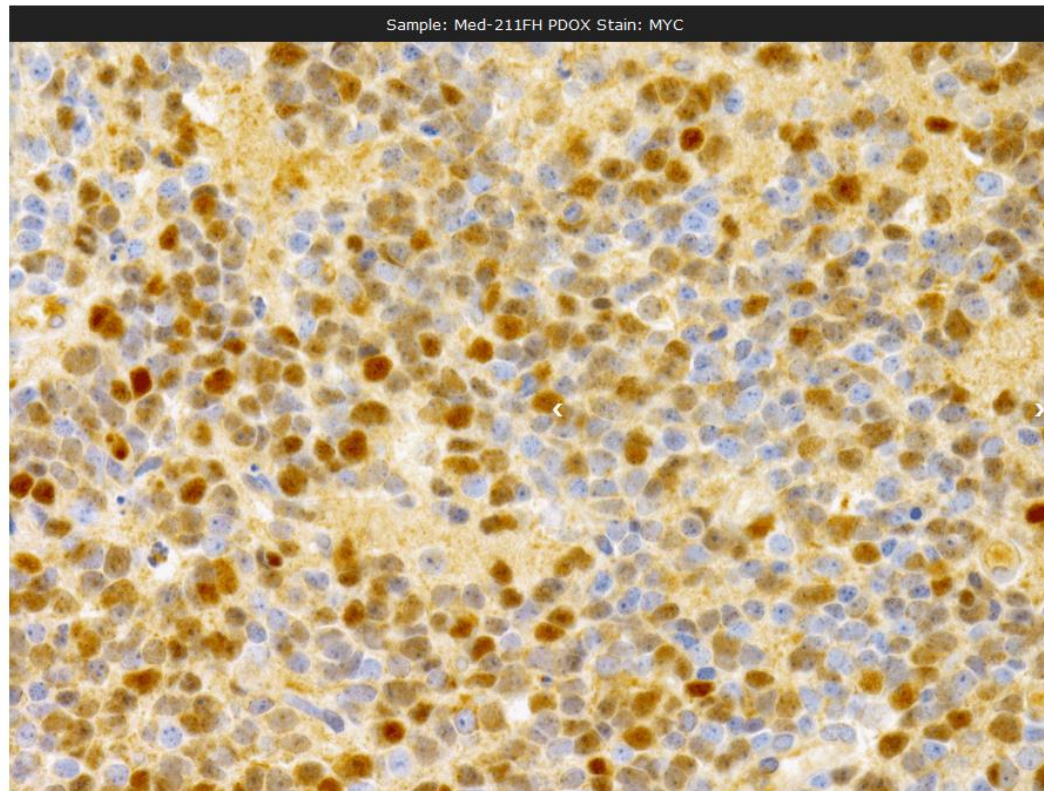
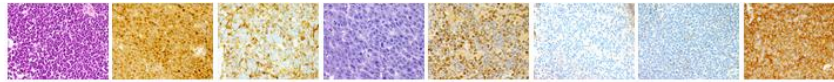
PDX Explorer: histology and IHC gallery



PDOX: H&E



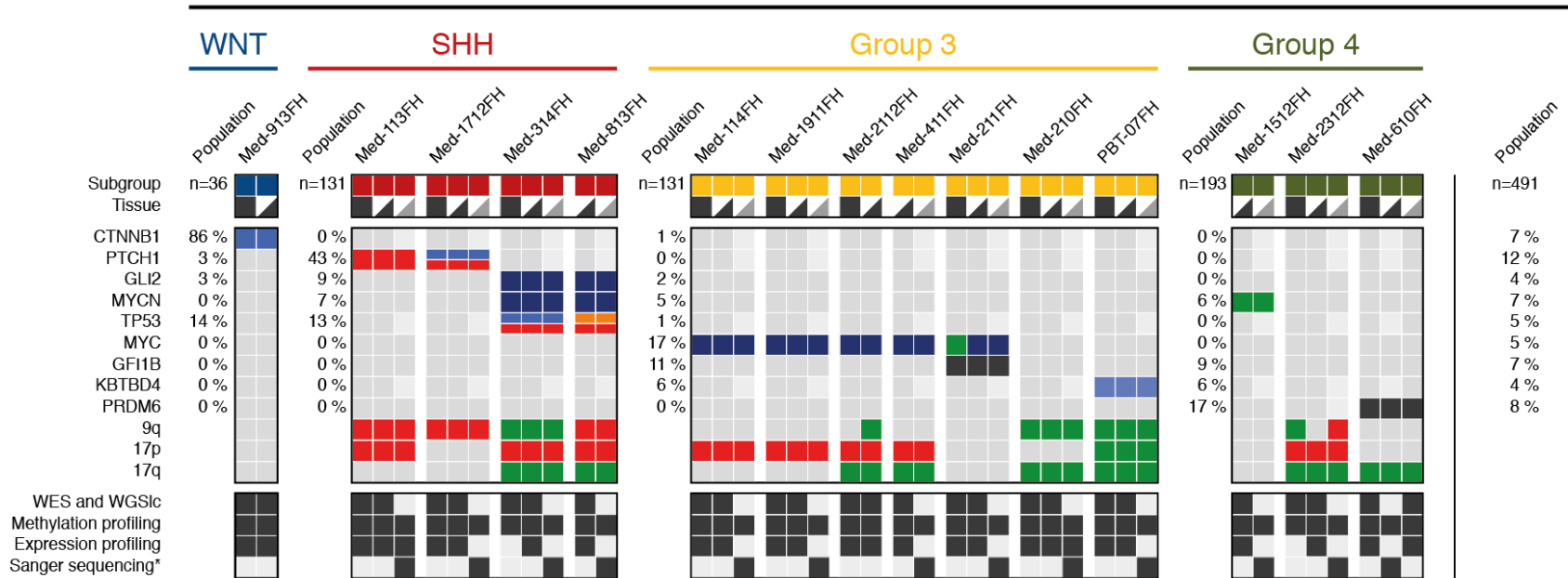
PDX Explorer: histology and IHC gallery



PDOX: IHC MYC

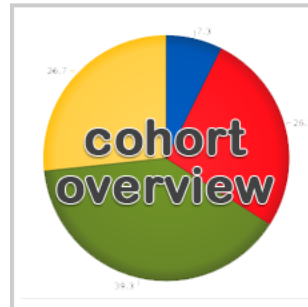
Oncoplots: Mutational landscape of MB PDOX

Medulloblastoma



PDOX models highly similar to their matching primary tumors
and stable across different passages

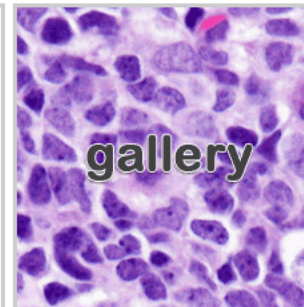
PDX explorer overview



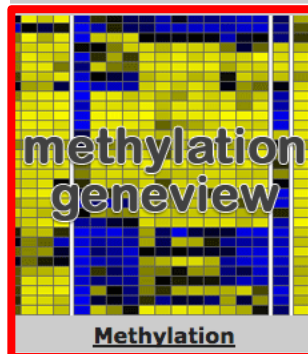
Cohort Overview



PDX Explorer



Histopathology



Methylation



Gene Expression



Circos Archive



Genome Browser

96	86199298	-	GT	G	exonic	RASSF9
	563260		G	A	exonic	RASSF7
76	48687677	rs146098580	C	T	exonic	ERAS
512	192973513	rs150310874	G	A	exonic	HRASL5
47	82366948		G	A	exonic	RASGEF18
48	79292149	rs141767671	G	A	exonic	RASGRF1
61	25378562	rs121913537	C	T	exonic	KRAS
83	25396		C	T	exonic	KRAS
83	25396		C	T	exonic	KRAS
546	141370545		T	A	exonic	RASA2
39	1556		T	A	exonic	RASA3
657	206766641		CA	C	exonic	RASSF5
26	82366727		C	T	exonic	RASGEF18
84	25398285	rs121913530	C	G	exonic	KRAS
743	115258744	rs121434596	C	T	exonic	NRAS
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747	115258748	rs121913250	C	T	exonic	NRAS

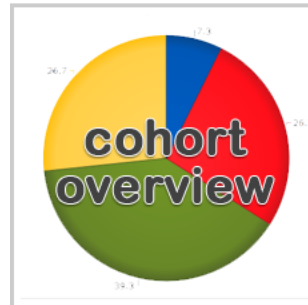
Somatic variants Table

Somatic Variants Table

1	A	B	C
1	100%	100%	100%
2	100%	100%	100%
3	100%	100%	100%
4	100%	100%	100%
5	100%	100%	100%
6	100%	100%	100%
7	100%	100%	100%
8	100%	100%	100%
9	100%	100%	100%
10	100%	100%	100%
11	100%	100%	100%
12	100%	100%	100%
13	100%	100%	100%
14	100%	100%	100%
15	100%	100%	100%
16	100%	100%	100%
17	100%	100%	100%
18	100%	100%	100%
19	100%	100%	100%
20	100%	100%	100%
21	100%	100%	100%
22	100%	100%	100%
23	100%	100%	100%
24	100%	100%	100%
25	100%	100%	100%
26	100%	100%	100%
27	100%	100%	100%

Additional Models

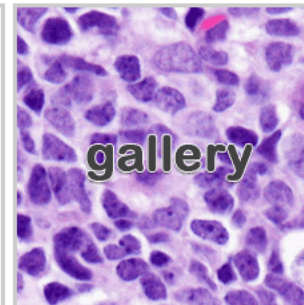
PDX explorer overview



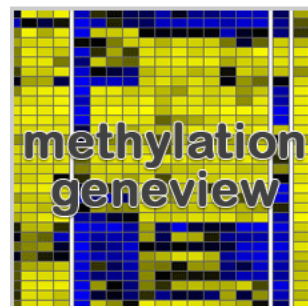
Cohort Overview



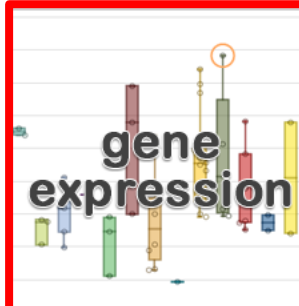
PDX Explorer



Histopathology



Methylation



Gene Expression



Circos Archive



Genome Browser

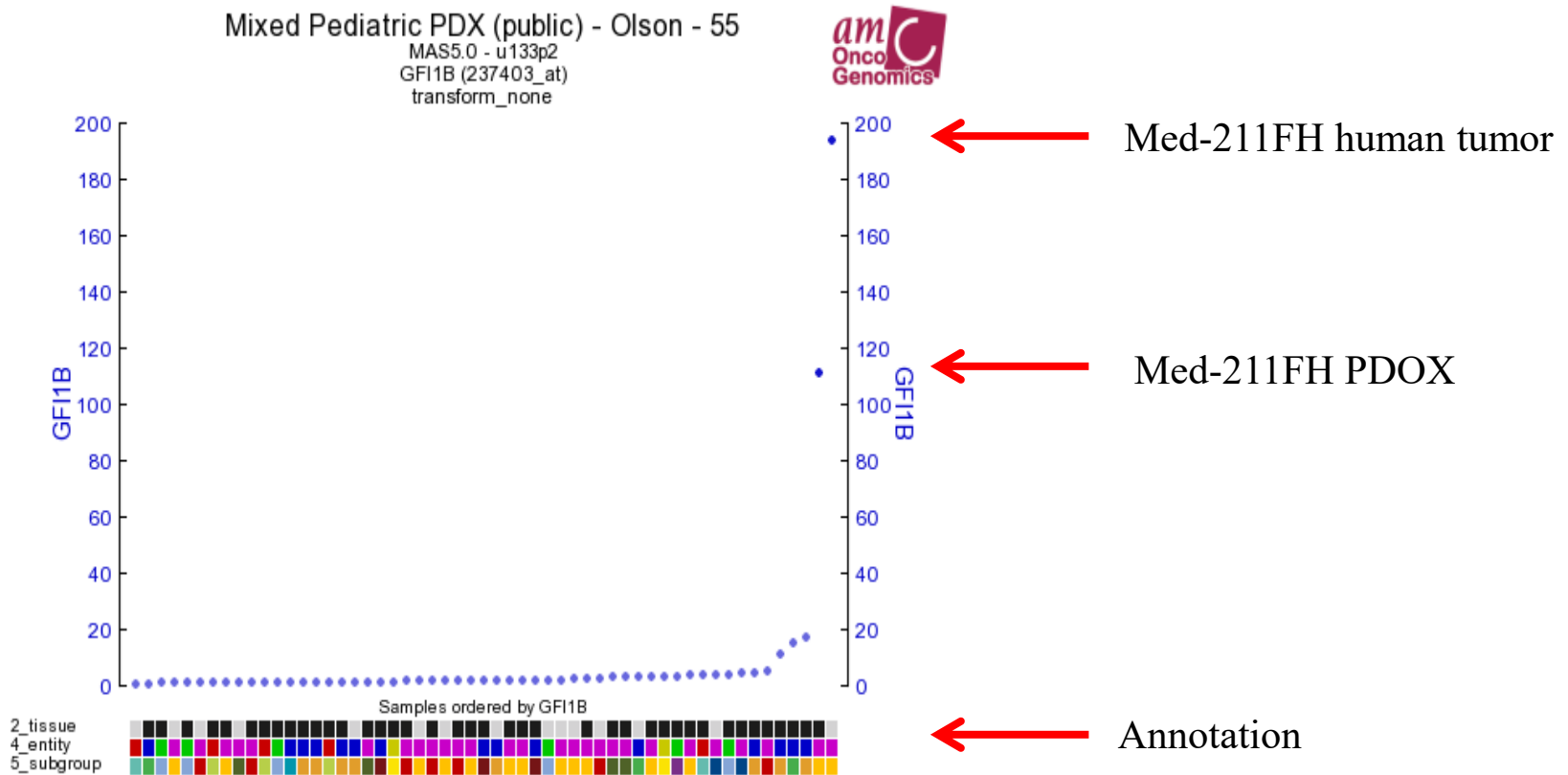
95	86199298	-	GT	G	exonic	RASSF9
	563260	-	G	A	exonic	RASSF7
76	48687677	rs146098180	C	T	exonic	ERAS
512	192973513	rs150318874	G	A	exonic	HRASL5
47	82366948	-	G	A	exonic	RASGEF1B
48	79292149	rs141767671	G	A	exonic	RASGRF1
61	25378562	rs121913127	C	T	exonic	KRAS
83	25396	-	C	T	exonic	KRAS
83	25396	rs121913127	C	T	exonic	KRAS
546	141370545	-	T	A	exonic	RASA2
39	1556	-	T	A	exonic	RASA3
657	206766641	-	CA	C	exonic	RASSF5
26	82366727	-	C	T	exonic	RASGEF1B
84	25398285	rs121913530	C	G	exonic	KRAS
743	115258744	rs121434396	C	T	exonic	NRAS
746	115258747	rs121913237	C	T	exonic	NRAS
747	115258748	rs121913250	C	T	exonic	NRAS

Somatic Variants Table

	A	B	C
1	Index	Model	Cell Line
2	K002_012	Stage of breast/melanoma	1084
3	K002_011	Stage of breast/melanoma	1084
4	K002_011	Stage of breast/melanoma	Prostate Form A
5	K002_011	Stage of breast/melanoma	Prostate Form A
6	K002_011	Stage of breast/melanoma	Prostate Form A
7	K002_011	Stage of breast/melanoma	Prostate Form A
8	K002_011	Stage of breast/melanoma	Prostate Form A
9	K002_011	Stage of breast/melanoma	Prostate Form A
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15	K002_011	Stage of breast/melanoma	Prostate Form A
16	K002_011	Stage of breast/melanoma	Prostate Form A
17	K002_011	Stage of breast/melanoma	Prostate Form A
18	K002_011	Stage of breast/melanoma	Prostate Form A
19	K002_011	Stage of breast/melanoma	Prostate Form A
20	K002_011	Stage of breast/melanoma	Prostate Form A
21	K002_011	Stage of breast/melanoma	Prostate Form A
22	K002_011	Stage of breast/melanoma	Prostate Form A
23	K002_011	Stage of breast/melanoma	Prostate Form A
24	K002_011	Stage of breast/melanoma	Prostate Form A
25	K002_011	Stage of breast/melanoma	Prostate Form A
26	K002_011	Stage of breast/melanoma	Prostate Form A
27	K002_011	Stage of breast/melanoma	Prostate Form A

Additional Models

PDX Explorer: Gene expression



Summary

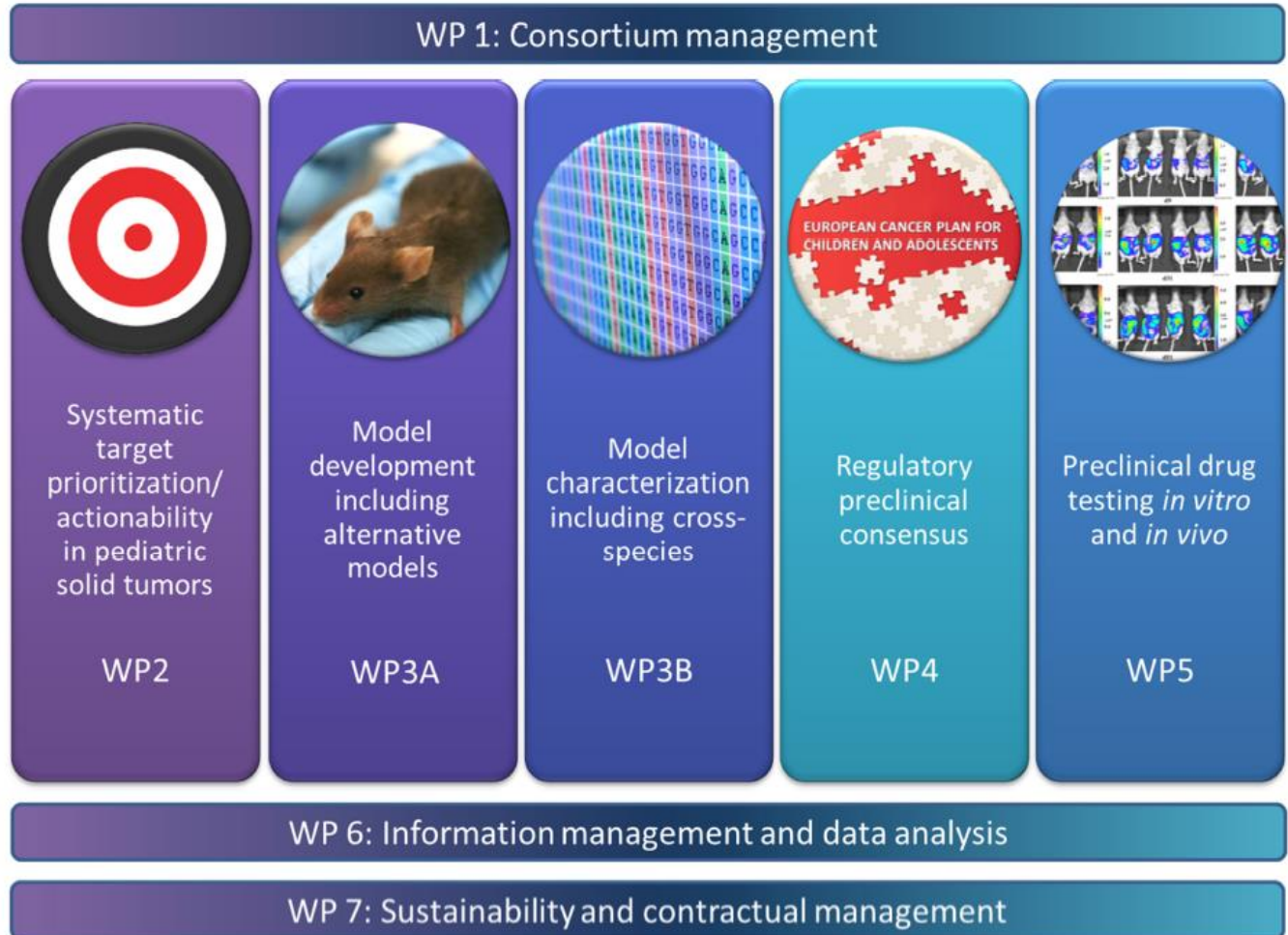
- **130 PDOX models** representing **22 distinct molecular types** of pediatric brain tumors have been generated and characterized in a joint effort by the pediatric neurooncology community
- Overrepresentation of most aggressive subtypes (MYC/N)
- **PDX explorer:** database for PDX models and associated molecular data
- All essential information is funneled into **PDX facts sheets**
- **Easier access to PDX and molecular data for the scientific community**

Outlook

- Add more models (HD, Houston, San Diego, **ITCC-P4**, and others?)
- Add additional layer of data, e.g. drug screening data
- Fine-tuning of existing platform
- Add pediatric PDX to existing PDX databases

ITCC-P4 - IMI-2 Consortium:

Generation and characterization of **>400 *in vitro* and *in vivo* (PDX) models** across **solid pediatric cancers** for preclinical studies. Broad collaboration between academia, SME's and Pharma. Proof of concept for immunotherapies in humanized models



ITCC-P4 current status (Feb 2020)

All tumor types
with pdx and total nr



rhabdomyosarcoma
with pdx and total nr



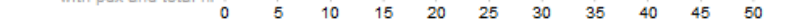
ewing sarcoma
with pdx and total nr



osteosarcoma
with pdx and total nr



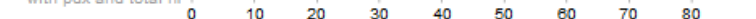
high grade glioma
with pdx and total nr



medulloblastoma
with pdx and total nr



neuroblastoma
with pdx and total nr



hepatoblastoma
with pdx and total nr



rhabdoid tumor
with pdx and total nr



ependymoma
with pdx and total nr



malignant nerve sheath tumor
with pdx and total nr



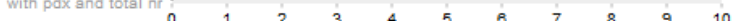
synovial sarcoma
with pdx and total nr



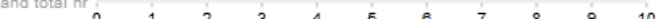
ETMR
with pdx and total nr



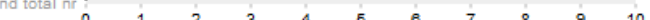
HGNET-BCOR
with pdx and total nr



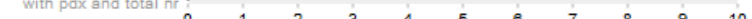
DSCRT
with pdx and total nr



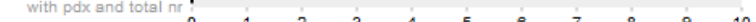
CCSK
with pdx and total nr



pineoblastoma
with pdx and total nr

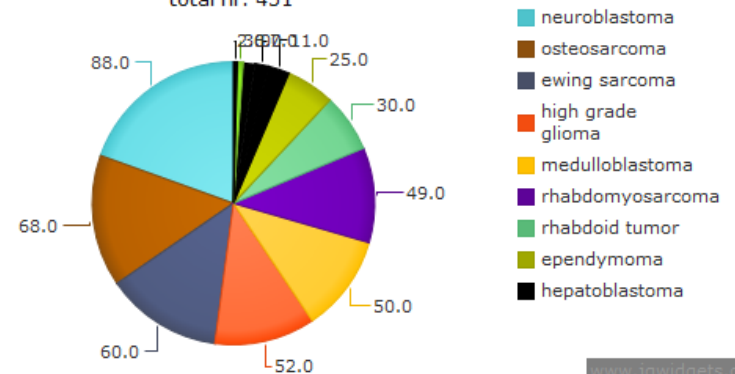


retinoblastoma
with pdx and total nr



ITCC-P4-Models

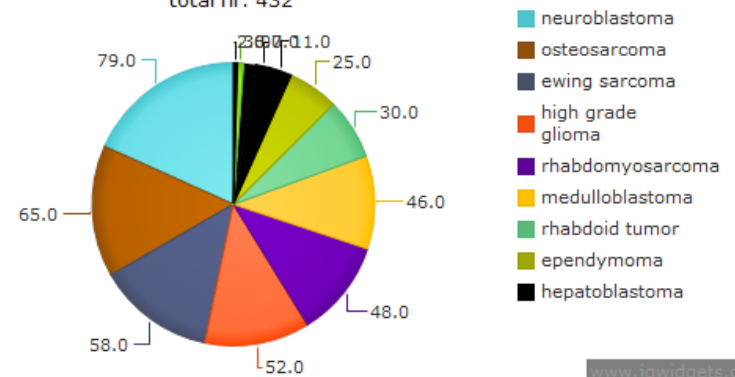
total nr: 451



www.iqwiggets.com

ITCC-P4-Models with PDX

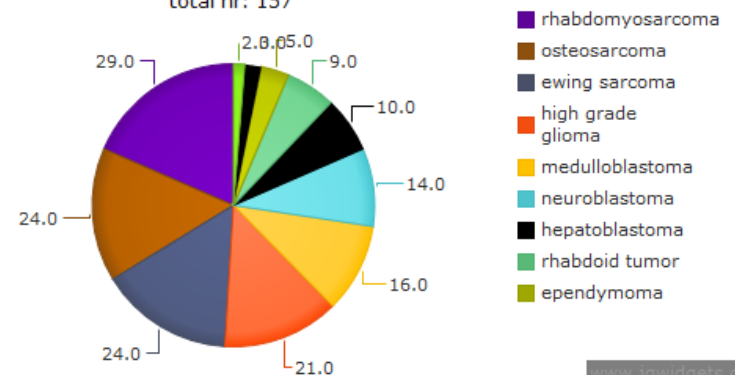
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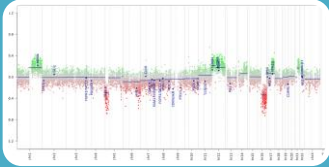
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Established ITCC-P4-Models

total nr: 157

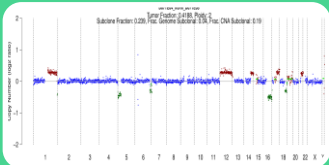


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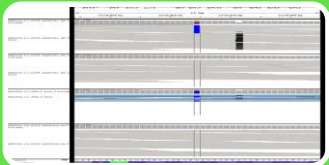
DNA methylation : Illumina EPIC or 850k bead arrays

- human (primary) tumor DNA
- tumor DNA of PDX model



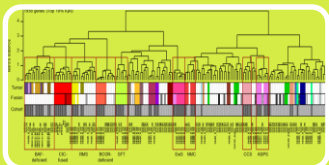
Low coverage (2-3x) WGS (Illumina)

- human germ line/normal healthy tissue DNA
- human (primary) tumor DNA
- tumor DNA of PDX model



High coverage (100 - 120x) WES (Illumina, SureSelect Human exon V5 kit or equivalent)

- human germ line/normal healthy tissue DNA
- human (primary) tumor DNA
- tumor DNA of PDX model



RNA sequencing (input: RNA RIN ≥ 7) strand-specific Illumina; Expected : 65M raw pair reads)

- human (primary) tumor DNA
- tumor DNA of PDX model



Affymetrix gene expression human Affymetrix 133plus2.0 arrays (input: RIN ≥ 7).

- human (primary) tumor DNA

In vivo testing

- All done at SMEs
- 40 different PDX models per entity
- 3 SOC drugs plus 6 targeted compounds per entity
- N = 1 mouse per PDX and per drug
- Readouts: tumor size and survival
- Correlate response with molecular data
- Biomarkers for stratification in clinical trial?

Many distinct molecular entities of brain tumors require multiple distinct model systems for preclinical studies

Model systems:

- Cell lines
 - Organoids 
 - GEMMs
 - In utero electroporation mouse models
 - PDOX models  including immunoprecient mice
- Cerebral organoids (mini brains)
 - Cerebellar organoids
 - Brain stem organoids
 - Brain tumor organoids
 - Organoid co-cultures

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