Molecular classification and characterization of pediatric brain tumors –

need for more and better preclinical models

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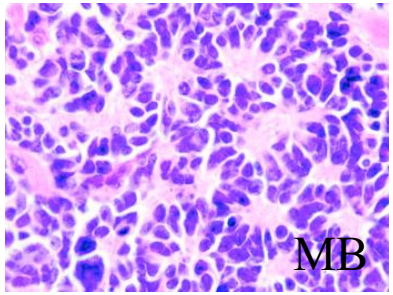
Edinburgh, February 27th, 2020

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

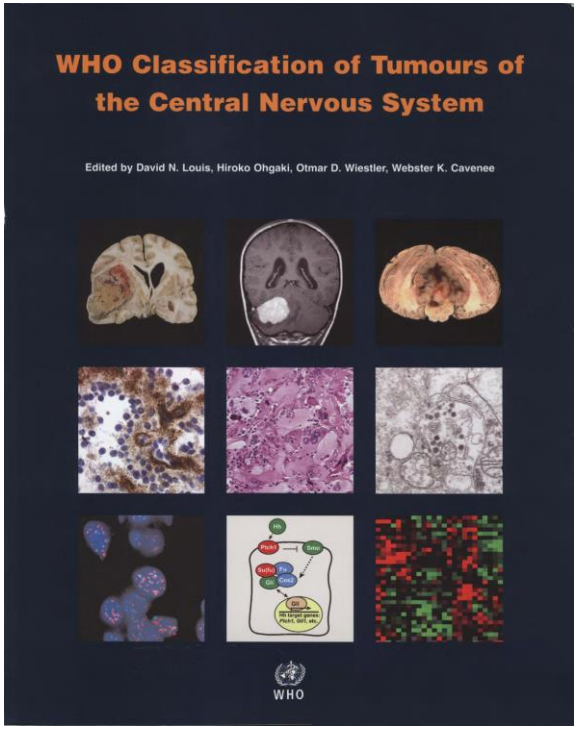
- Meningioma (n = 15!)
- Mesenchymal tumors
- Melanocytic tumors

Germ Cell Tumors

- Germinoma
- Teratoma

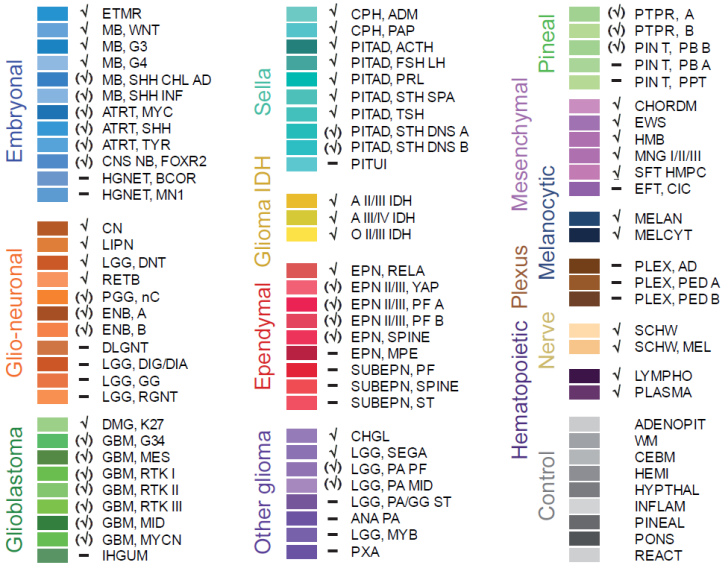
Sellar Tumors

- Craniopharyngioma
- Pituicytoma



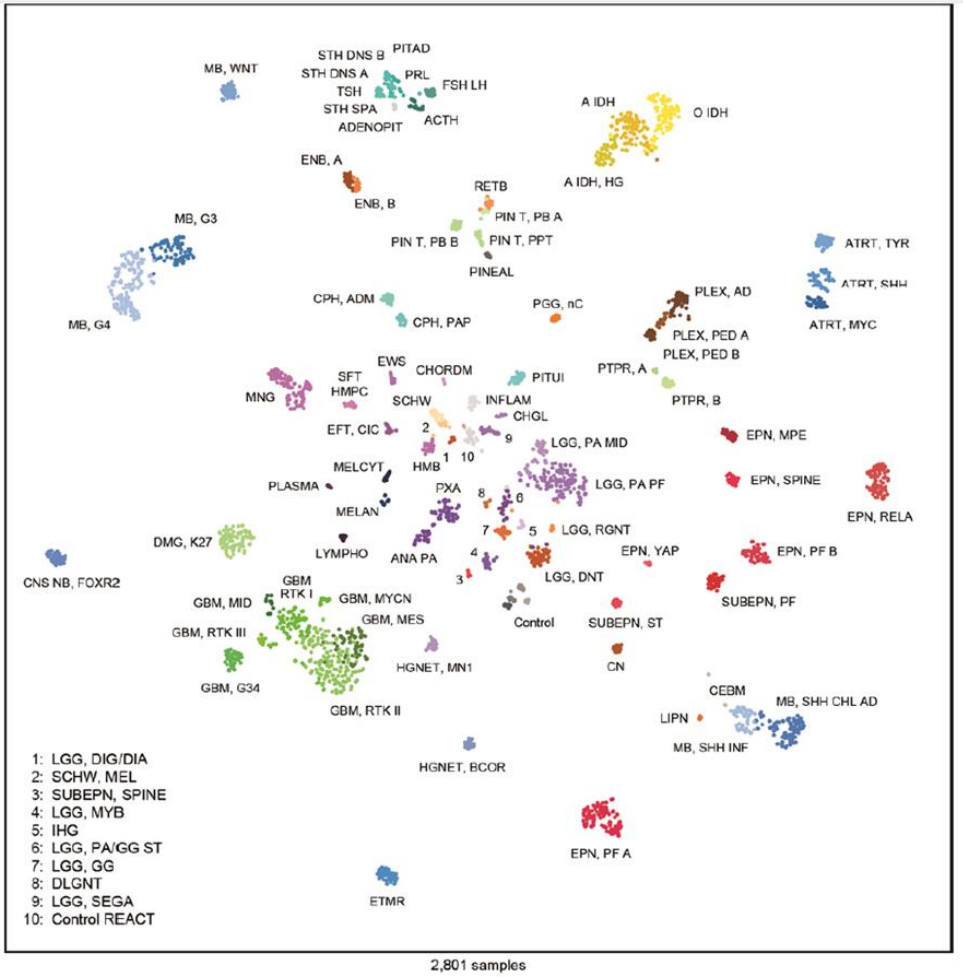
Using DNA methylation for classification purposes

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



Identification of at least 82 distinct molecular subtypes of CNS tumors

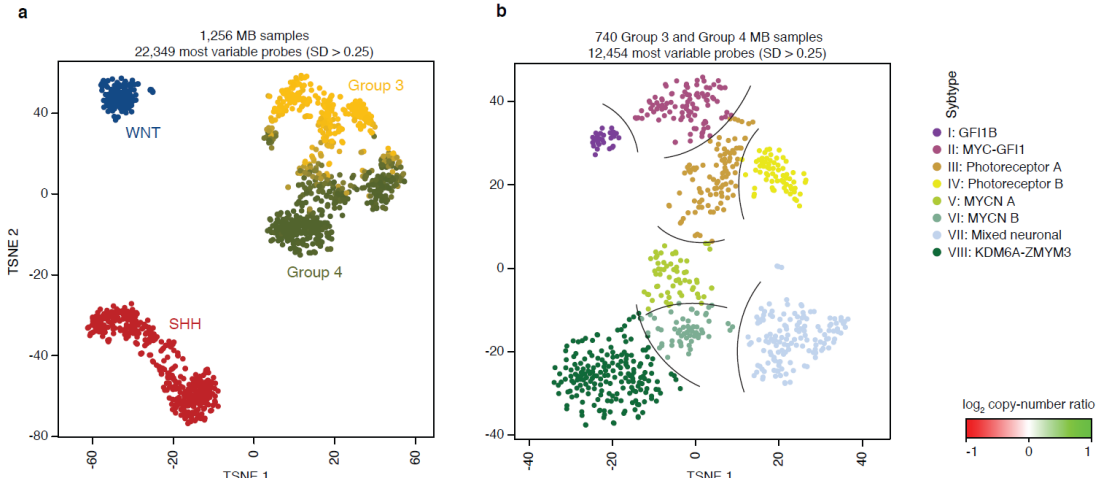
Update will contain >170 distinct subtypes!



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

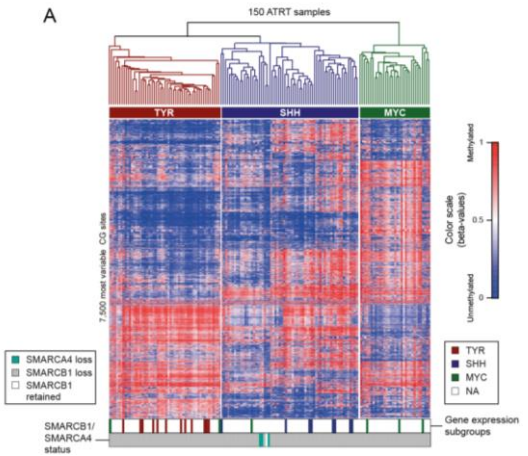
Molecular classification examples:

medulloblastoma

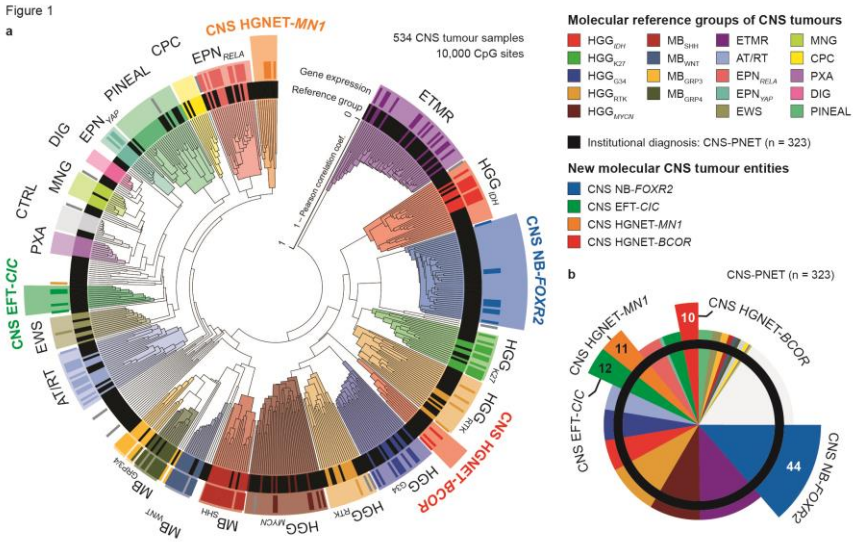


Northcott et al., Nature 2017

ATRT

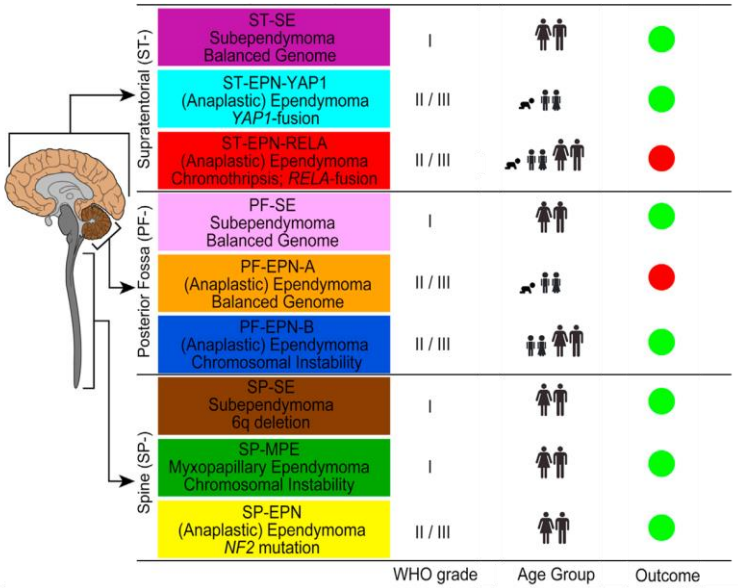


Johann et al., Cancer Cell 2016
Erkek et al., Cancer Cell 2019



CNS-PNETs

Sturm et al., Cell 2016

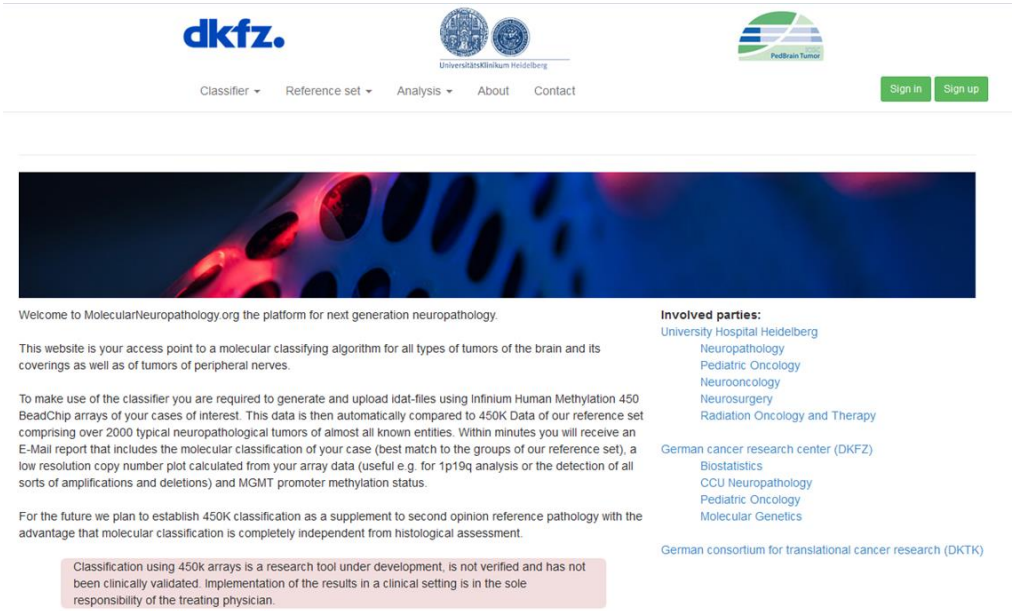


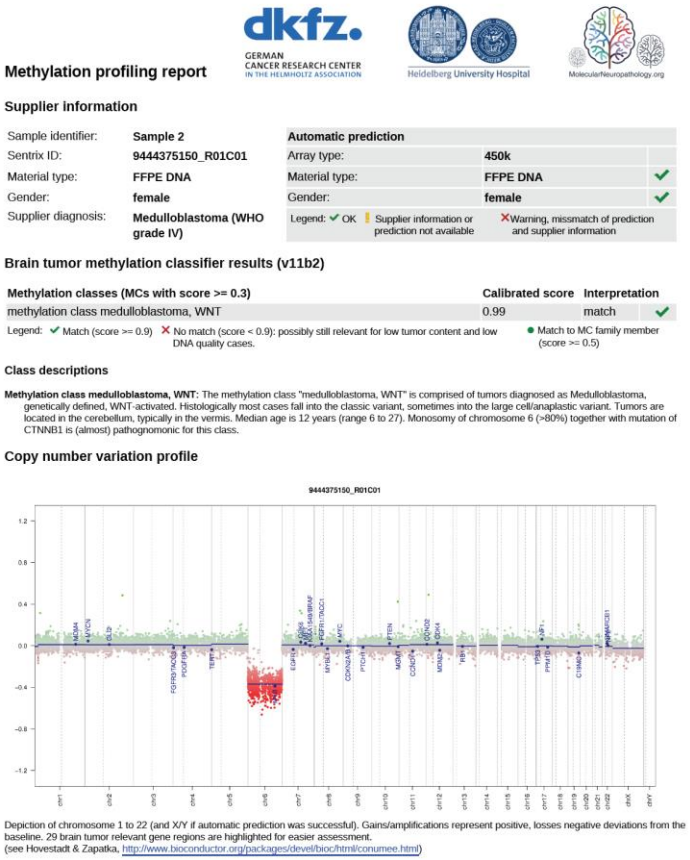
ependymoma

Pajtler et al., Cancer Cell 2015

Methylation classifier website

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



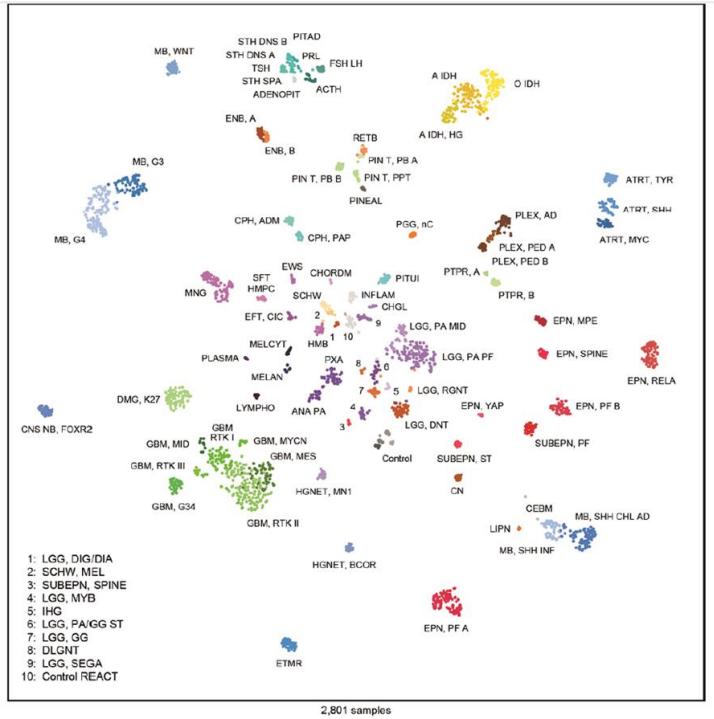


Already >37000 external profiles analyzed!

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**

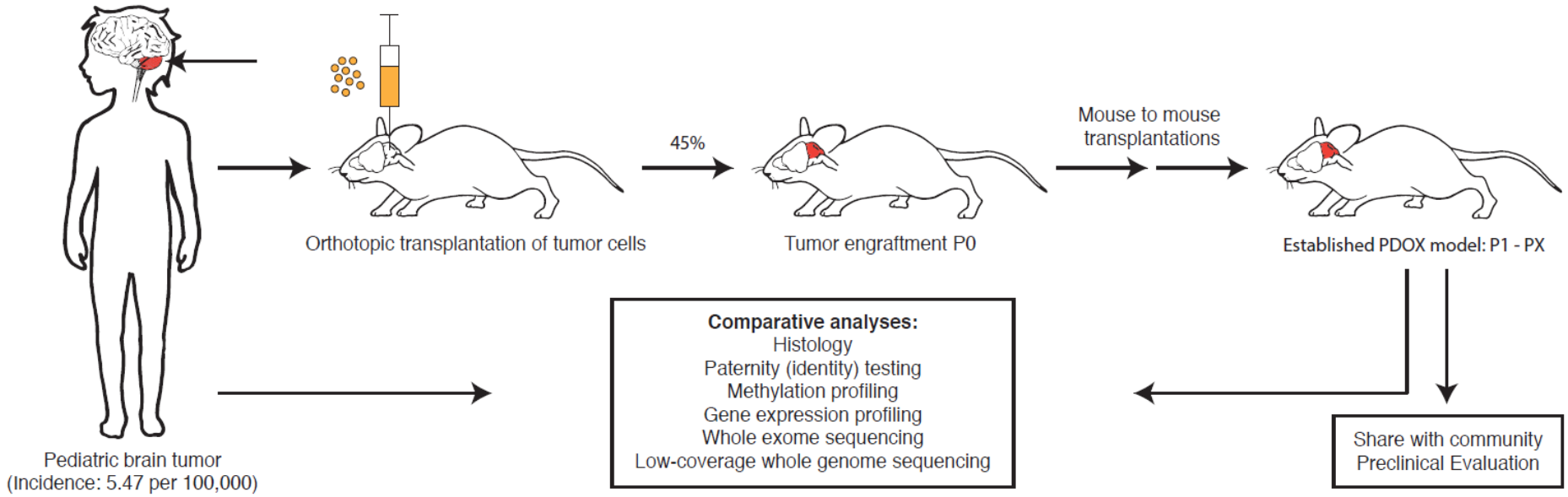
Collaborative Approach between different labs:

- 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



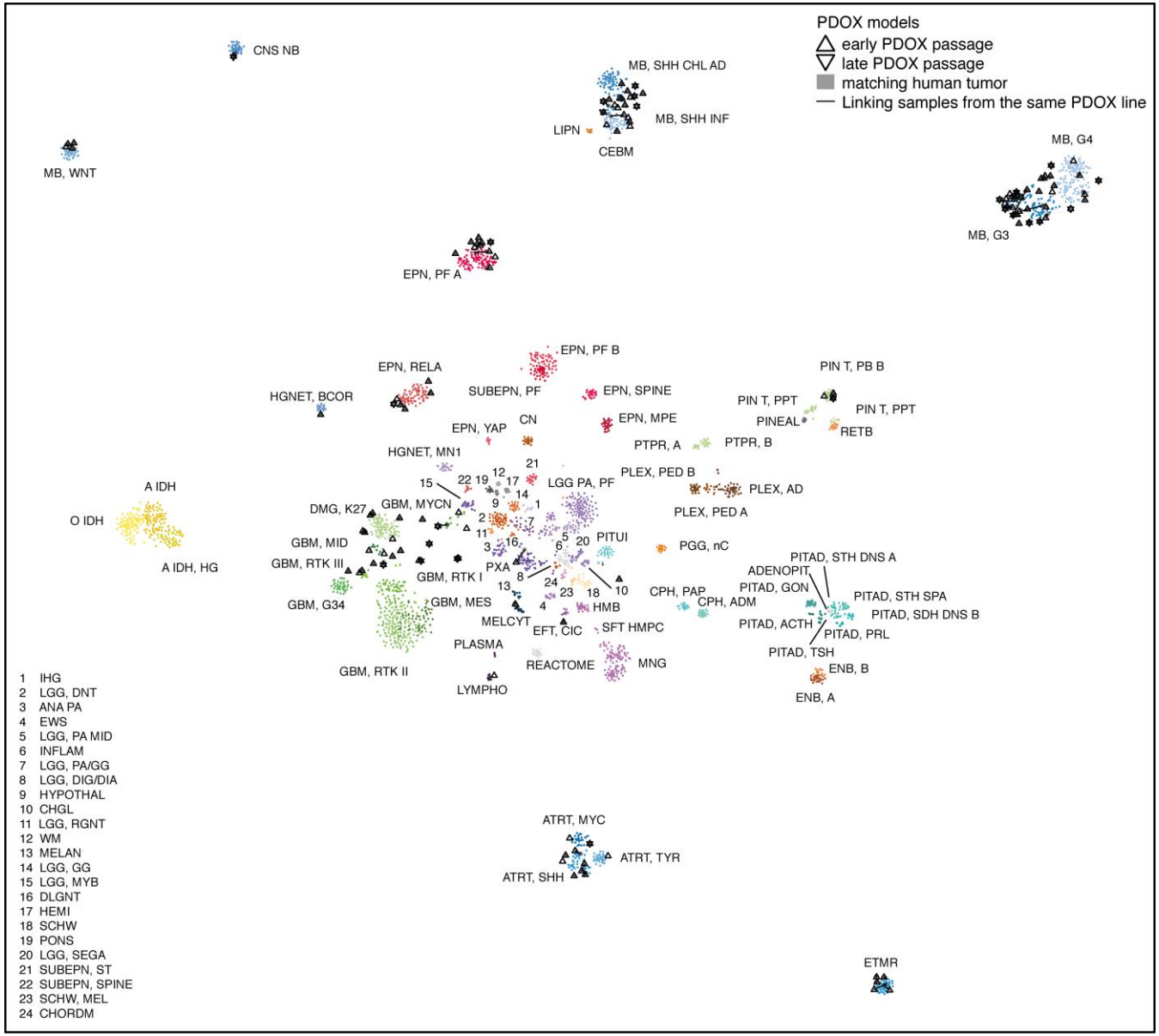
Brabetz et al., Nature Medicine 2018

Molecular Characterization (PDOX, primary tumor, blood):

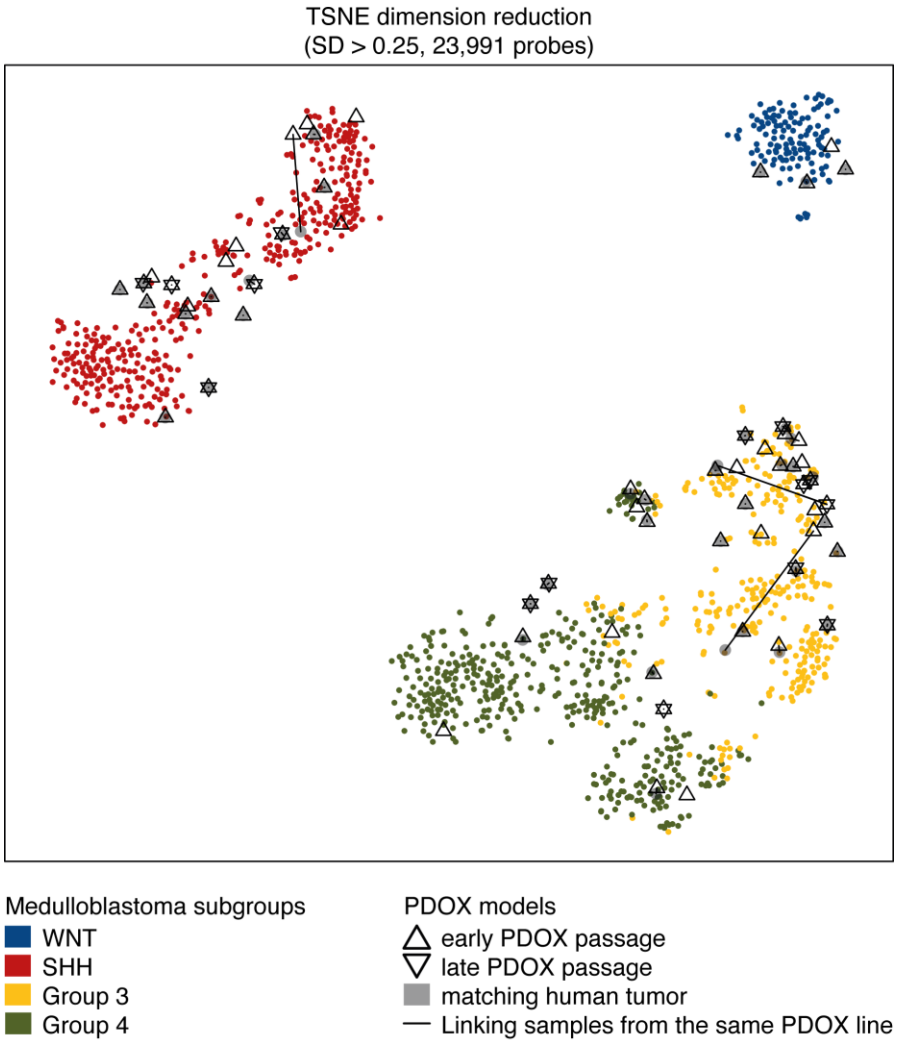
- 450K Methylation Profiling: Tumor Classification, CNVs
- Gene Expression Profiling: pathways, drug targets
- Whole Exome Sequencing: SNVs / indels
- Low coverage WGS: SVs, CNVs

Currently >130 PDOX models fully analyzed

Molecular classification of 130 brain tumor PDOX

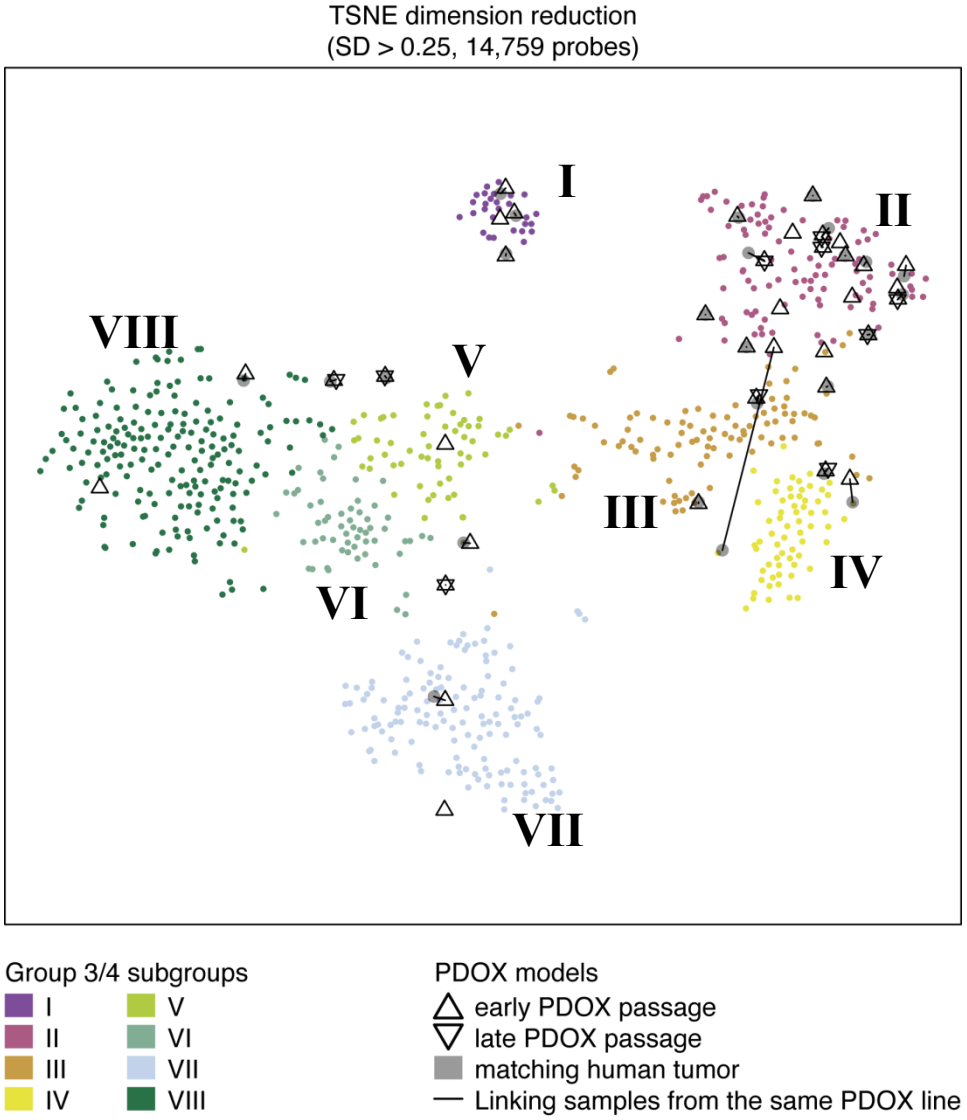


Molecular classification of medulloblastoma PDOX (n = 62)



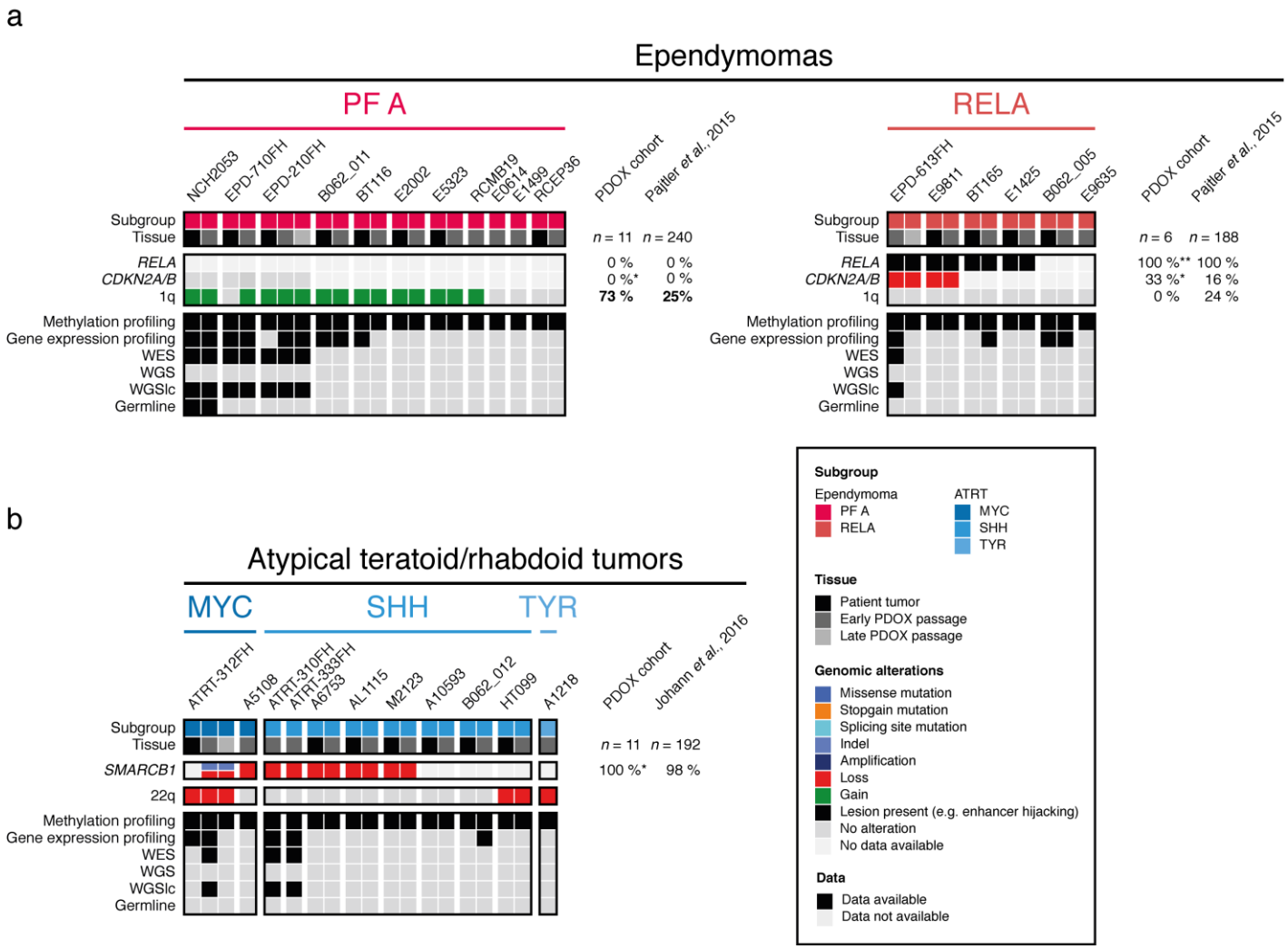
MB WNT:	**4**
MB SHH:	**21**
MB GRP3:	**24**
MB GRP4:	**13**

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



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

Bias for most aggressive tumors even within tumor subgroups



PDX data sheets and PDX Explorer: Making data available

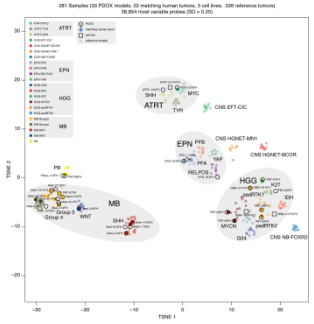
Brabetz , Leary et al., Nature Medicine, 2018

How to make the data accessible?

How information is usually shared:

Figures in paper + supplementary material

Raw data: EGA + GEO

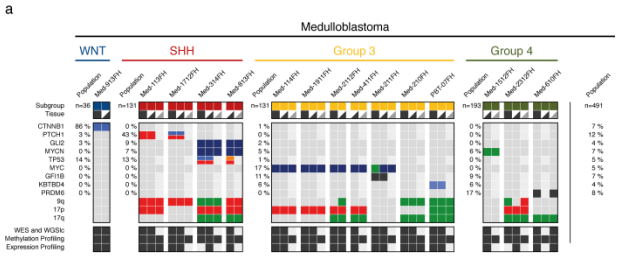


S1-S23

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:

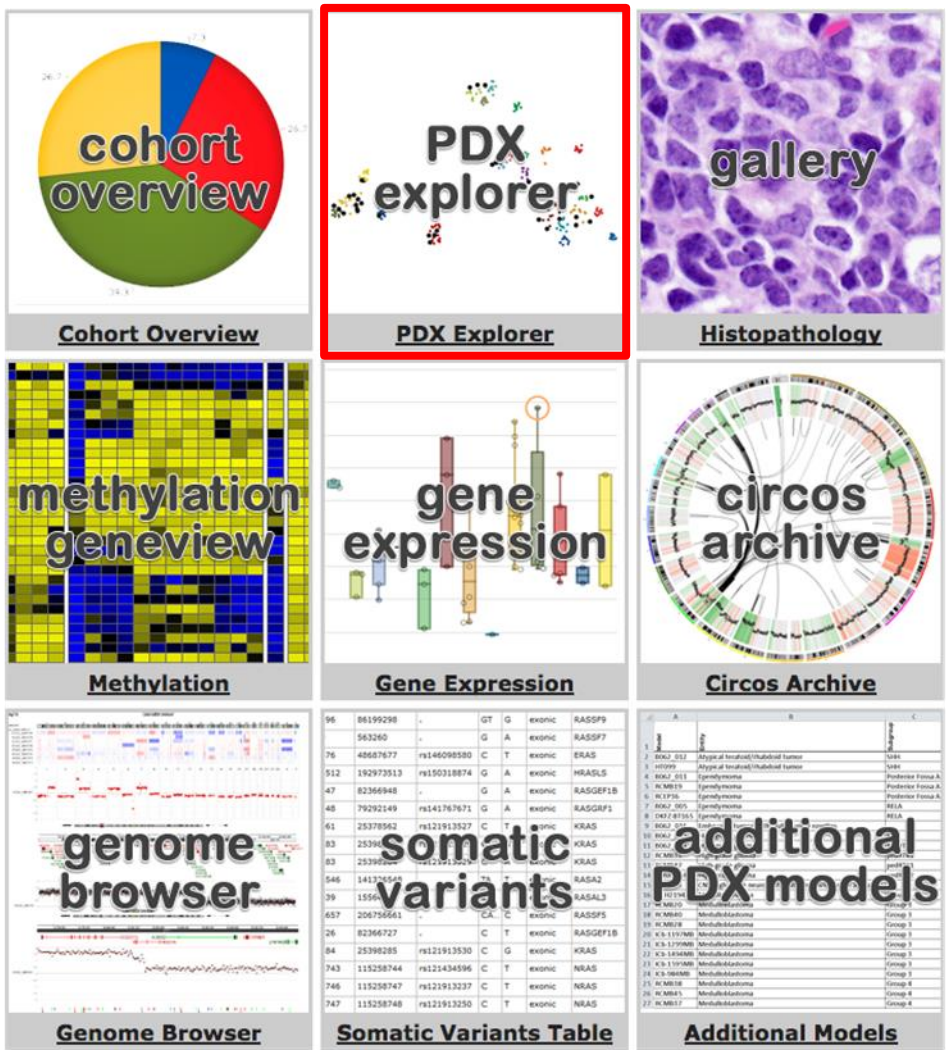
PDX data sheets

PDX Explorer in R2 (r2.amc.nl)

Easy ordering of lines via www.btrl.org

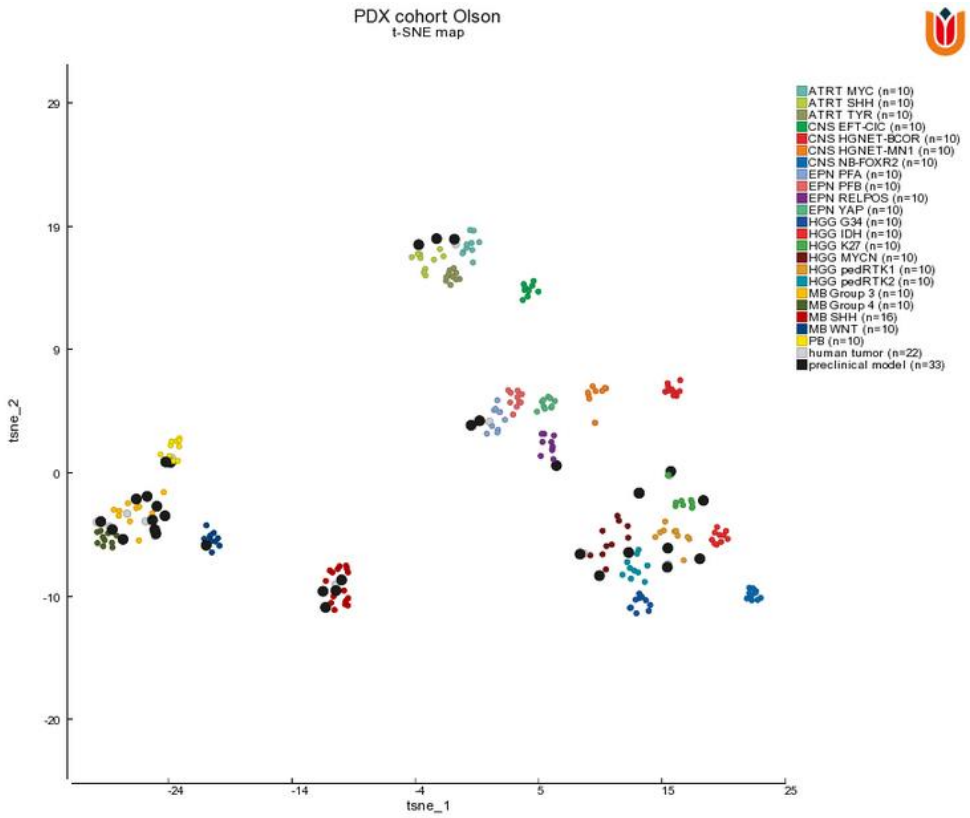
Brabetz , Leary et al., Nature Medicine 2018

PDX Explorer (r2.amc.nl)



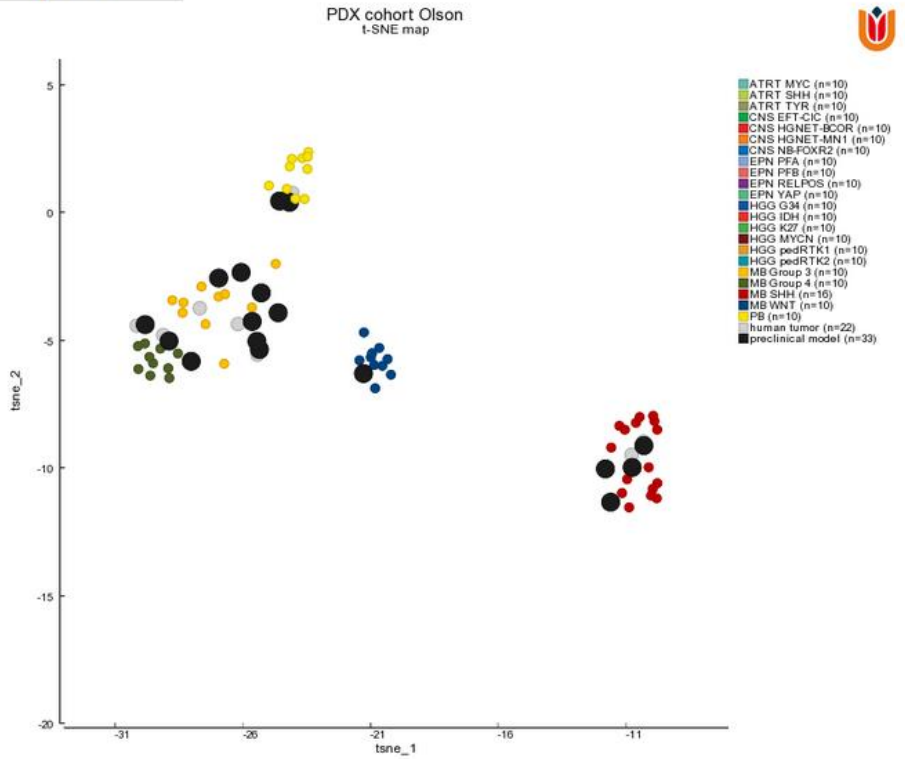
In collaboration with Jan Koster, AMC Amsterdam, the Netherlands

PDX Explorer



Directly Explore Molecular Data

Explorer



MB

mb_grp3	mb_grp4	mb_shh	mb_wnt
med-114fh	med-1512fh	med-113fh	med-913fh
med-1911fh	med-2312fh	med-1712fh	
med-210fh	med-610fh	med-314fh	
med-2112fh		med-813fh	
med-211fh			
med-411fh			
pbt-07fh			

Fact sheet

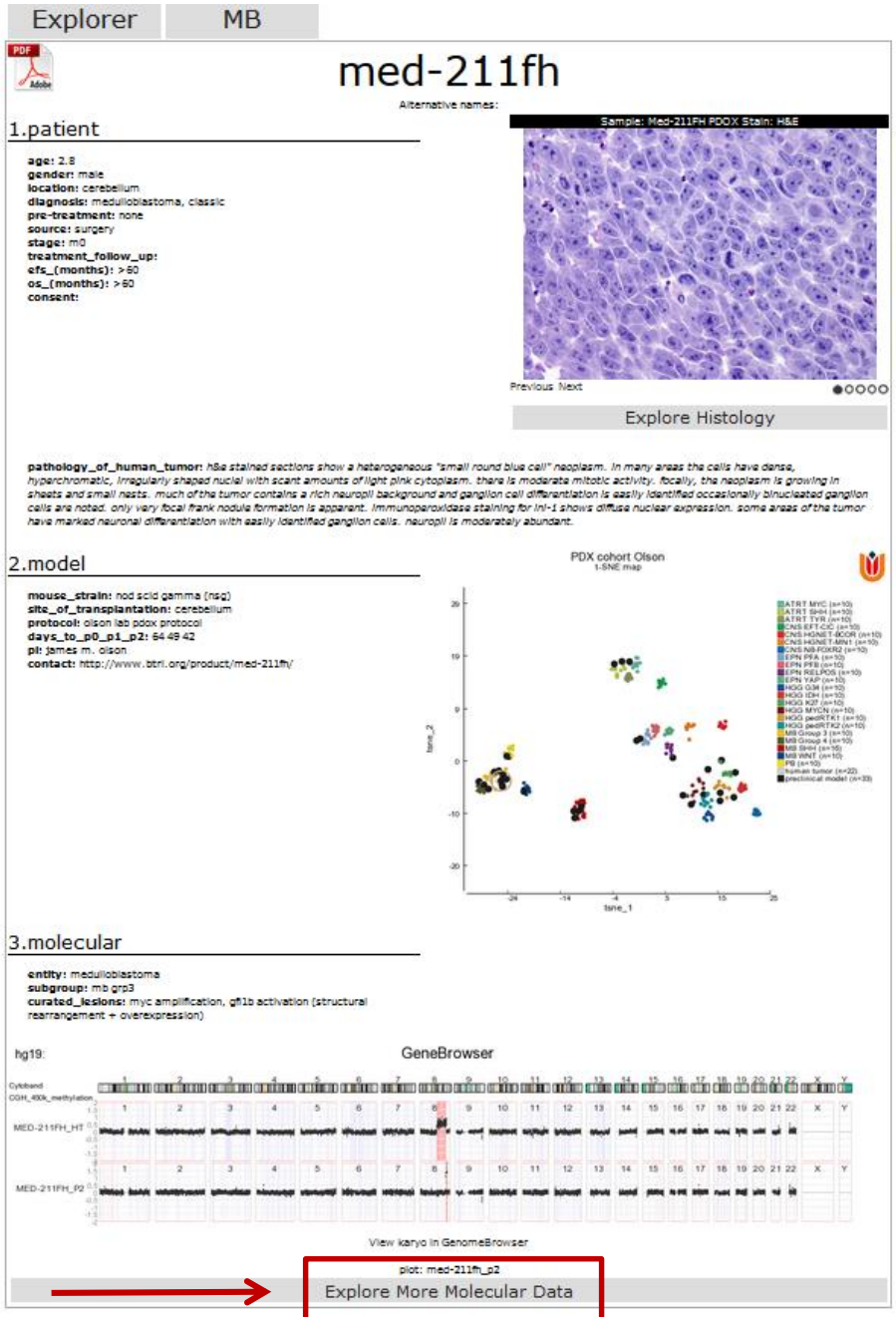
Download PDF

Patient information

Model information

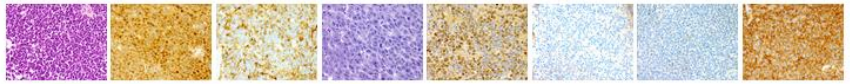
Molecular data

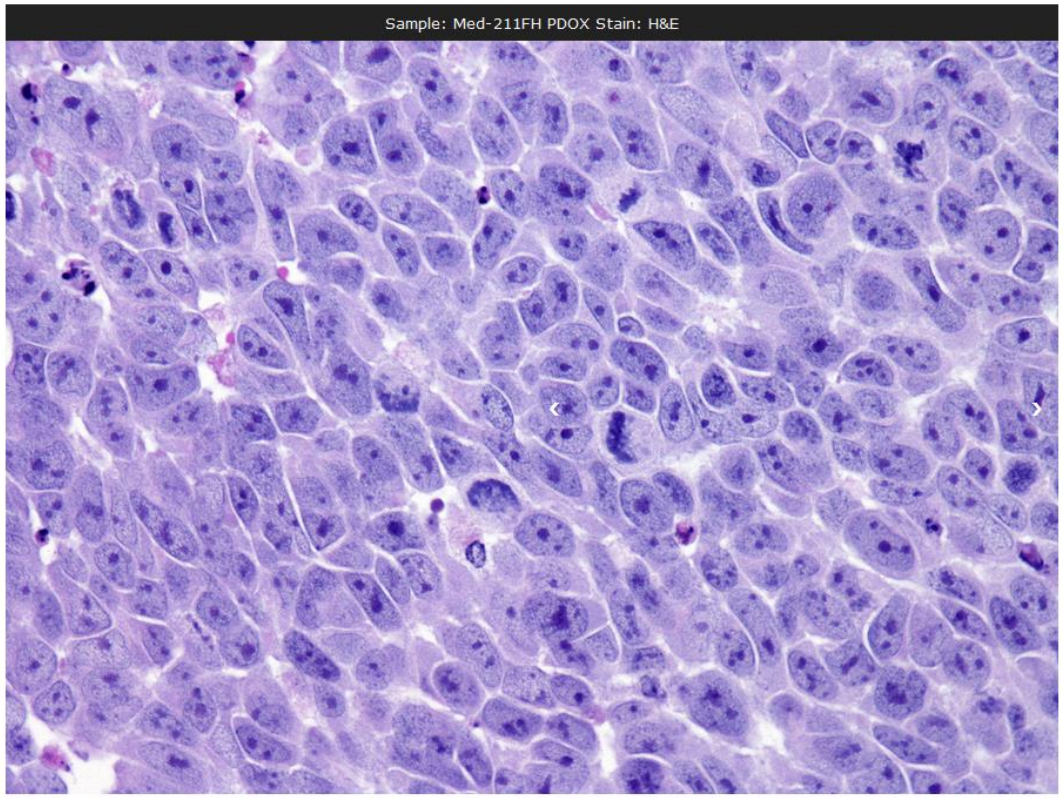
In depth molecular data



Histology and IHC gallery

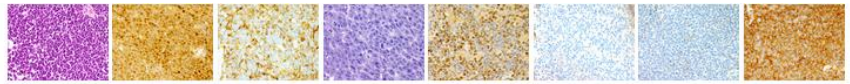
PDX Explorer: histology and IHC gallery

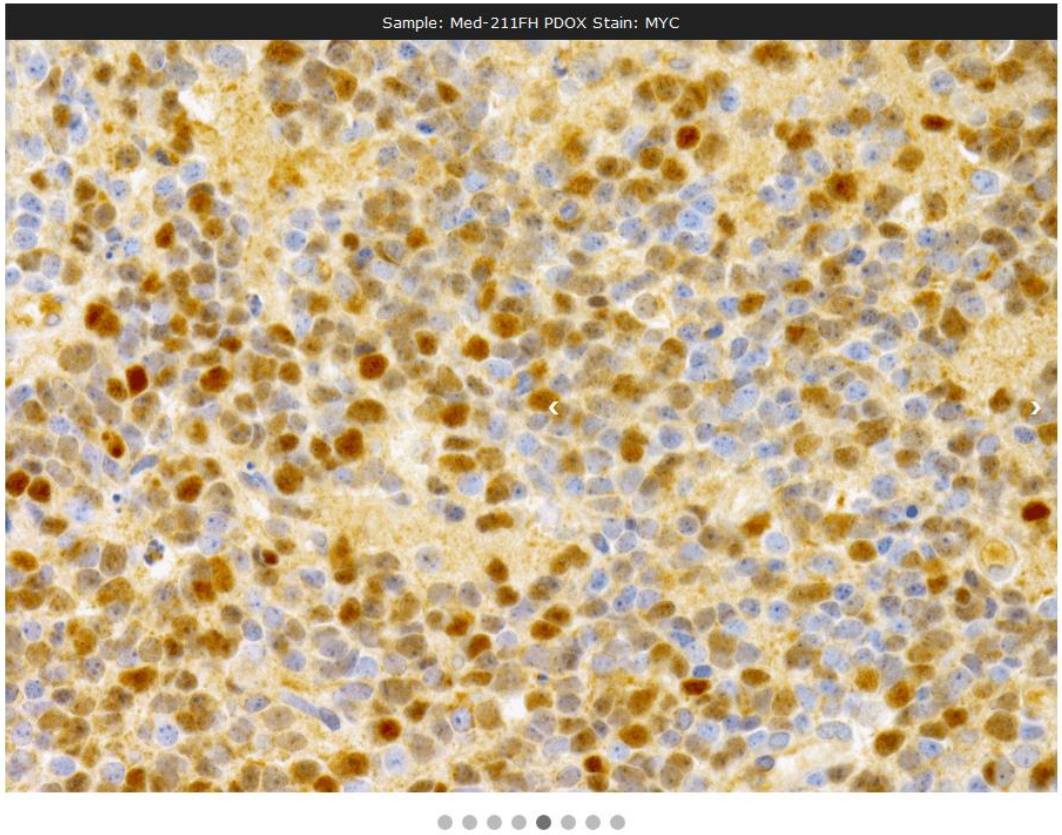




PDOX: H&E

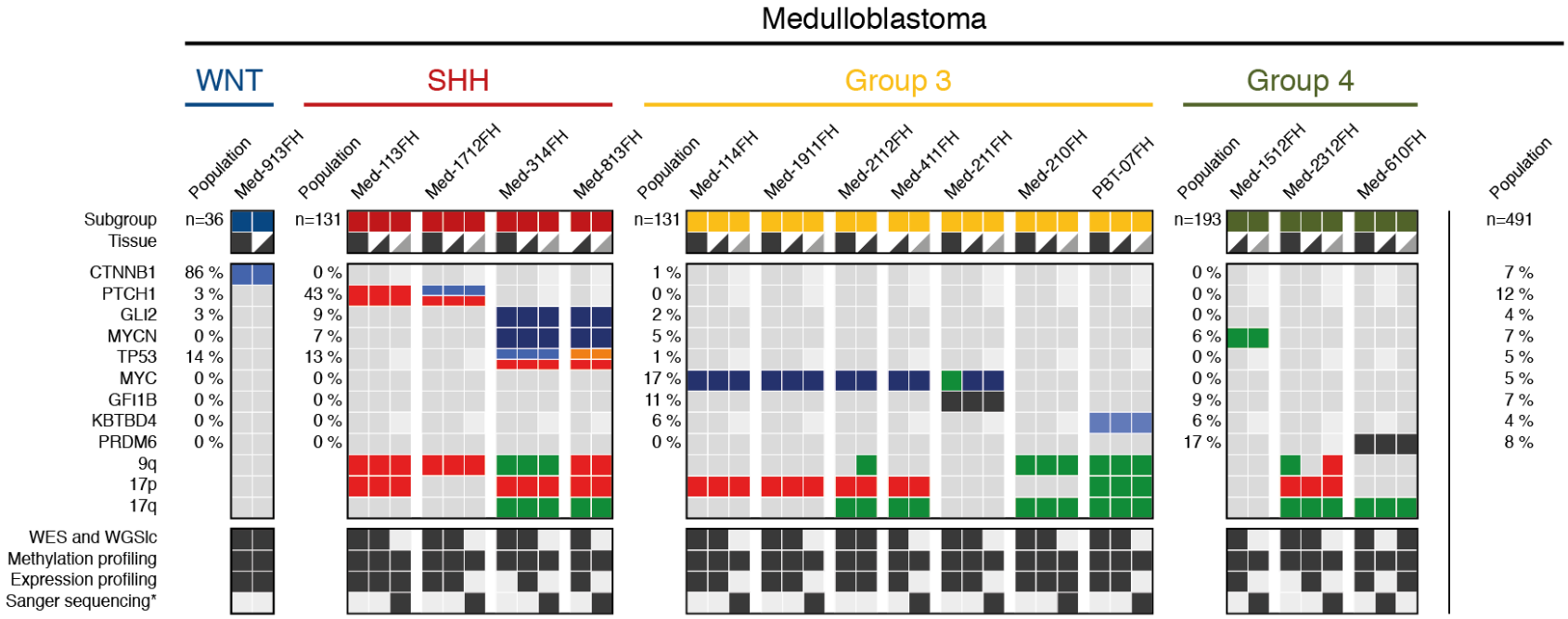
PDX Explorer: histology and IHC gallery





PDOX: IHC MYC

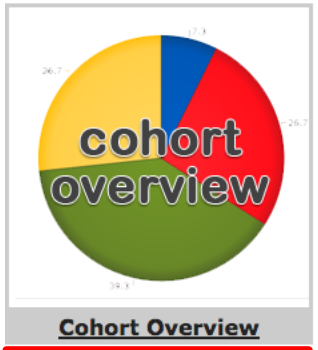
Oncoplots: Mutational landscape of MB PDOX



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

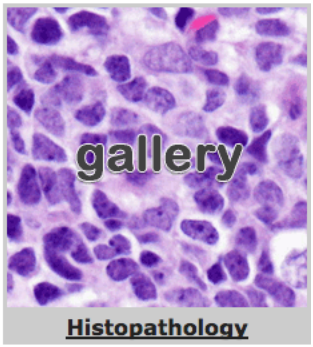
Brabetz , Leary et al., Nature Medicine, 2018

PDX explorer overview

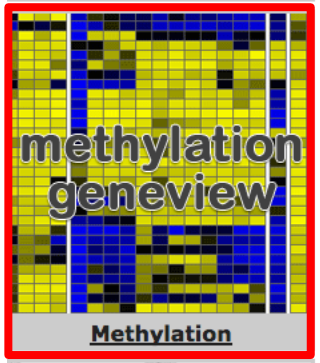


Cohort Overview

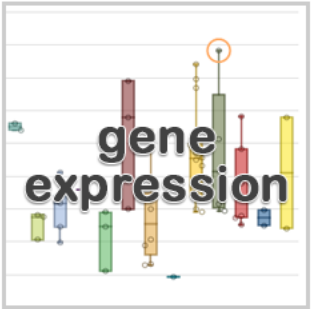
PDX Explorer



Histopathology



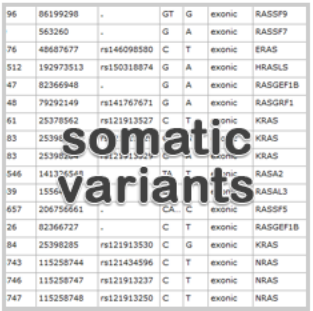
Methylation



Gene Expression

Circos Archive

Genome Browser



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	563260	.	G	A	exonic	RASSF7
76	48687677	rs146098580	C	T	exonic	ERAS
512	192973513	rs150318874	G	A	exonic	HRASLS
47	82366948	.	G	A	exonic	RASGEF1B
48	79292149	rs141767671	G	A	exonic	RASGRF1
61	25378562	rs121913527	C	T	exonic	KRAS
83	25398					KRAS
83	25398					KRAS
546	141					RASA2
39	155					RASAL3
657	206756661	.	CA	C	exonic	RASSF5
26	82366727	.	C	T	exonic	RASGEF1B
84	25398285	rs121913530	C	G	exonic	KRAS
743	115258744	rs121434596	C	T	exonic	NRAS
746	115258747	rs121913237	C	T	exonic	NRAS
747	115258748	rs121913250	C	T	exonic	NRAS

Somatic Variants Table

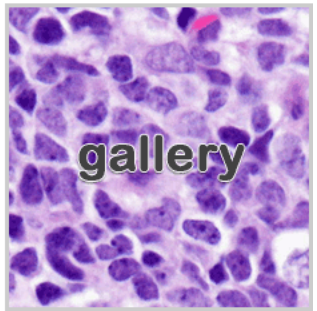
	A	B	C
1	Model	Entity	Subgroup
2	BOG_012	Atypical teratoid/rhabdoid tumor	SHH
3	HT099	Atypical teratoid/rhabdoid tumor	SHH
4	BOG_011	Ependymoma	Posterior Fossa A
5	RCMB19	Ependymoma	Posterior Fossa A
6	RCEP36	Ependymoma	Posterior Fossa A
7	BOG_005	Ependymoma	RELA
8	DKFZ-BT165	Ependymoma	RELA
9	BOG_		
10	BOG_		
11	BOG_		
12	RCMB		
13			
14			
15			
16	HD194		
17	RCMB10	Medulloblastoma	Group 3
18	RCMB40	Medulloblastoma	Group 3
19	RCMB28	Medulloblastoma	Group 3
20	KS-119/MB	Medulloblastoma	Group 3
21	KS-127/MB	Medulloblastoma	Group 3
22	KS-149/MB	Medulloblastoma	Group 3
23	KS-159/MB	Medulloblastoma	Group 3
24	KS-98/MB	Medulloblastoma	Group 3
25	RCMB38	Medulloblastoma	Group 4
26	RCMB45	Medulloblastoma	Group 4
27	RCMB17	Medulloblastoma	Group 4

Additional Models

PDX explorer overview

Cohort Overview

PDX Explorer



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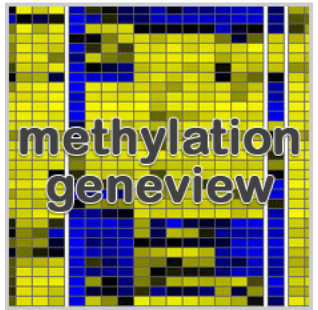

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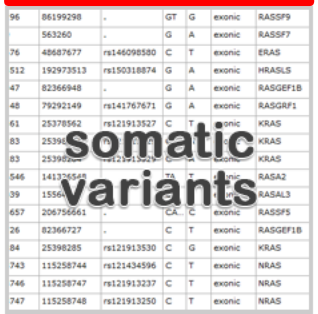

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8	DKFZ-BT165	Ependymoma	RELA
9	BOG_010	Ependymoma	RELA
10	BOG_		
11	BOG_		
12	RCMB		
13			
14			
15			
16	HD194		
17	RCMB10	Medulloblastoma	Group 3
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19	RCMB28	Medulloblastoma	Group 3
20	KS-119/MB	Medulloblastoma	Group 3
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24	KS-98/MB	Medulloblastoma	Group 3
25	RCMB38	Medulloblastoma	Group 4
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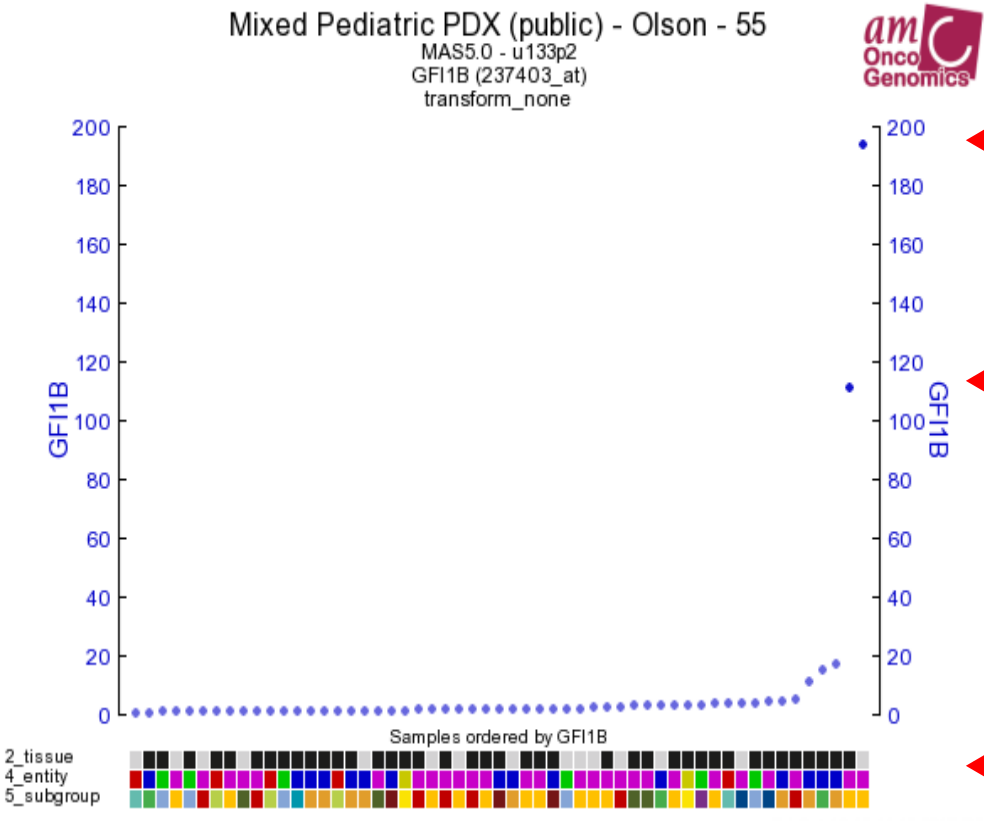
27	RCMB17	Medulloblastoma	Group 4

Additional Models

PDX Explorer: Gene expression



Med-211FH human tumor

Med-211FH PDOX

Annotation

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)

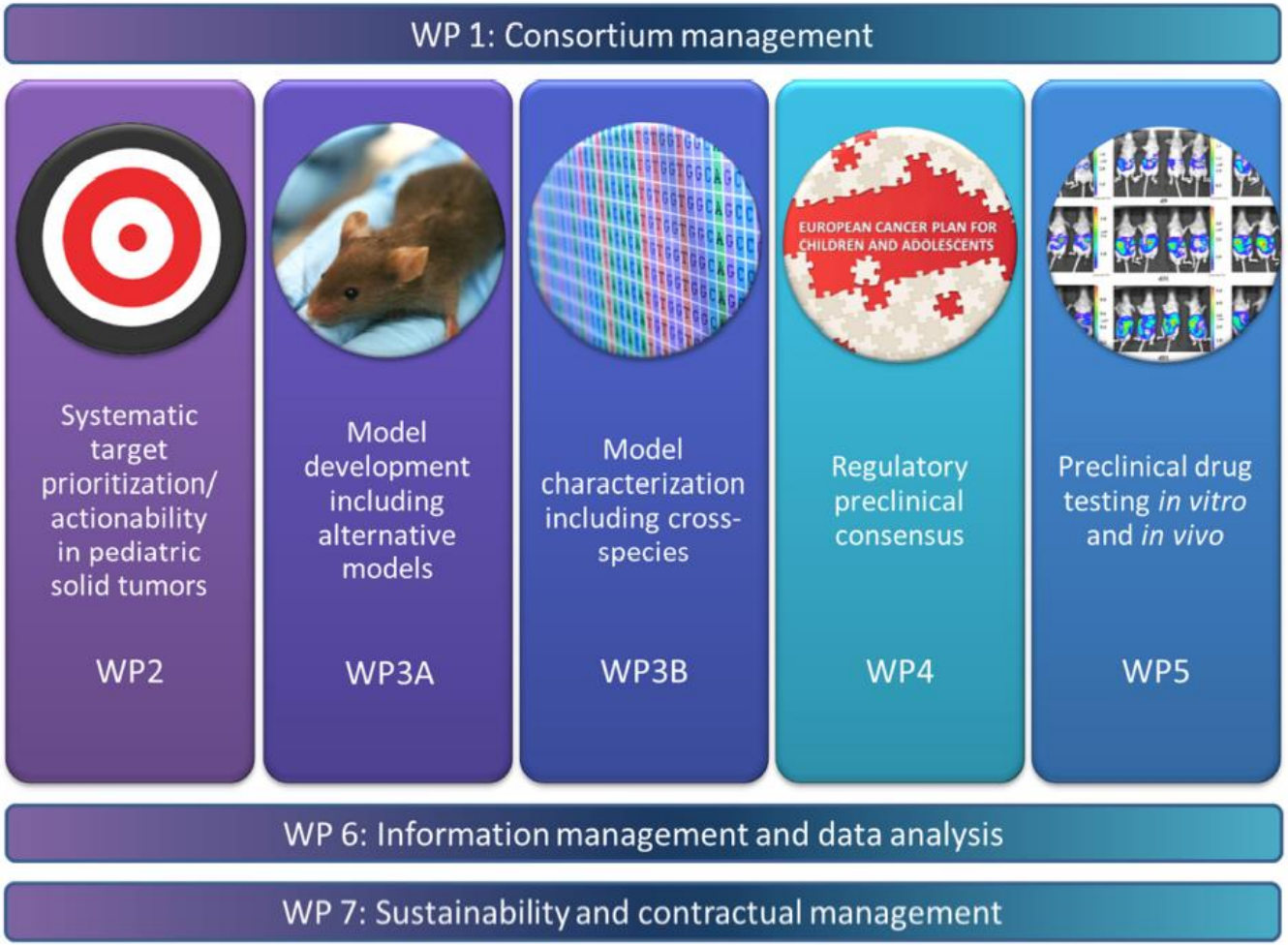
- **PDX explorer:** database for PDX models and associated molecular data
- All essential information is funneled into **PDX facts sheets**
- **Easier access to PDX and molecular data for the scientific community**

Outlook

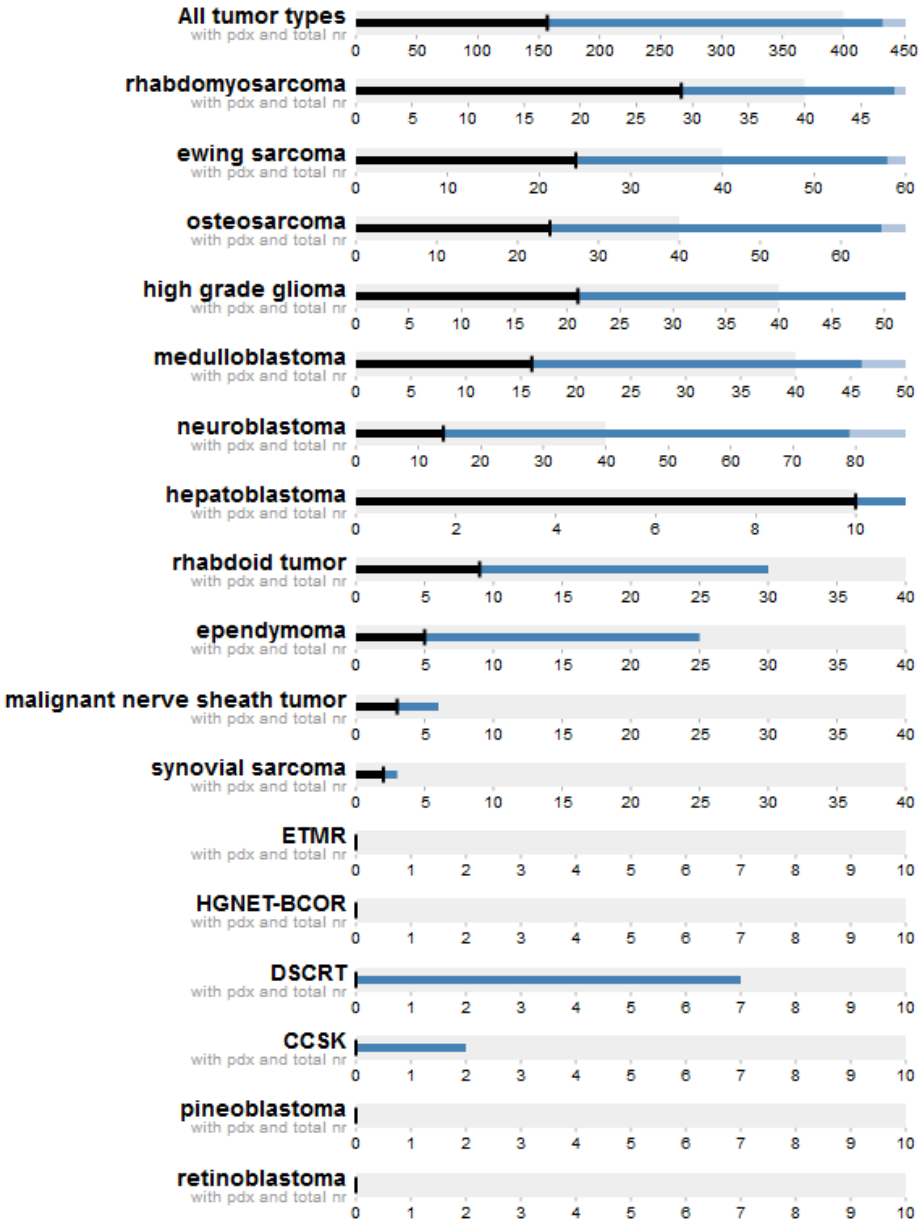
- Add more models (HD, Houston, San Diego, **ITCC-P4**, and others?)
- Add additional layer of data, e.g. drug screening data
- Fine-tuning of existing platform
- Add pediatric PDX to existing PDX databases

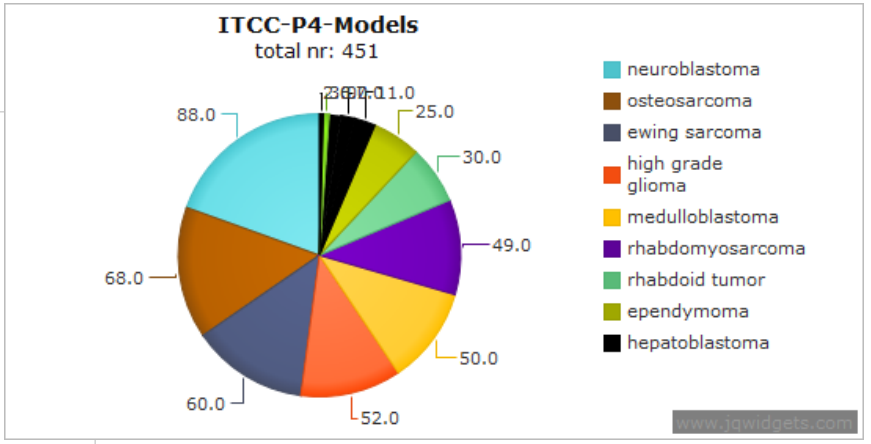
ITCC-P4 - IMI-2 Consortium:

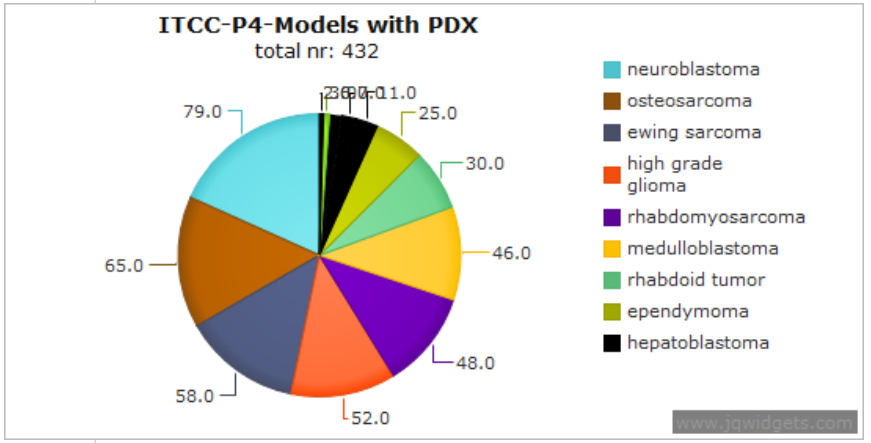
Generation and characterization **of >400 *in vitro* and *in vivo (PDX)* models** across **solid pediatric cancers** for preclinical studies. Broad collaboration between academia, SME's and Pharma. Proof of concept for immunotherapies in humanized models

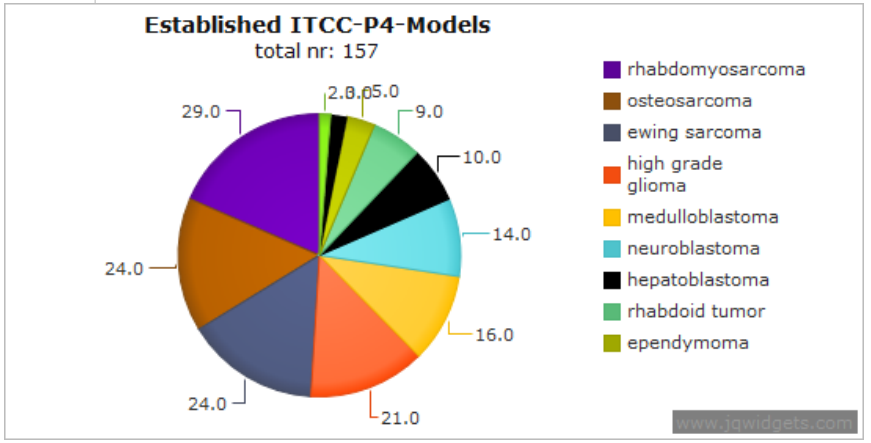


ITCC-P4 current status (Feb 2020)







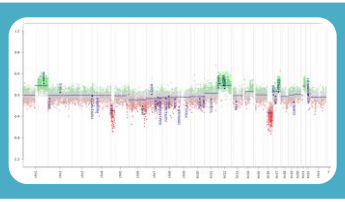


ITCC P4 PAEDIATRIC PRECLINICAL PROOF OF CONCEPT PLATFORM

IMI2 ITCC P4 WP3

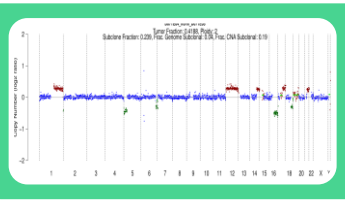
imi innovative medicines initiative

efpia



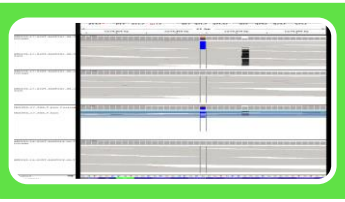
DNA methylation : Illumina EPIC or 850k bead arrays

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



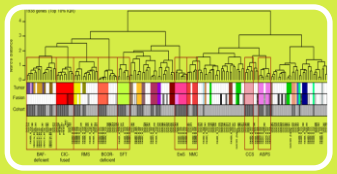
Low coverage (2-3x) WGS (Illumina)

- 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

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under Grant Agreement 116064. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and the European federation of Pharmaceutical Industries and Associations.
http://www.imi.europa.eu/

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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?

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http://www.imi.europa.eu/

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

Model systems:

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

Acknowledgements:

KiTZ
Hopp Children's Cancer Center
at the NCT Heidelberg


Sebastian Brabetz


Christin Schmidt


Susanne Gröbner


Norman Mack


Benjamin Schwalm

DKFZ:

Till Milde
Florian Selt
Jonas Ecker
Aniello Federico
Pascal Johann
Kristian Pajtler
Sander Lambo
Sjoerd van Rijn
Jens-Martin Hübner
Martin Sill
Natalie Jäger
David Jones
Christel Herold-Mende
Andrey Korshunov
Olaf Witt
Stefan Pfister

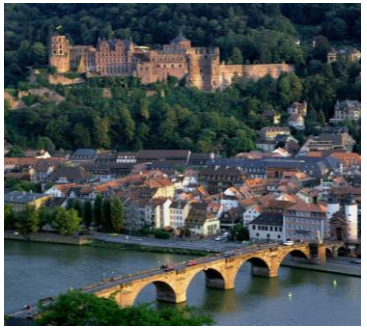
Collaborators:

Jim Olson, Sarah Leary, Madison Wise, Andy Strand, et al. (Seattle)
Rob Wechsler-Reya, Jessica Rusert, Alexandra Garancher,
James Jensen, Pablo Tamayo, Jill Mesirov (San Diego)
Xiao-Nan Li, Frank Braun, et al. (Houston)
Walter Berger, Johannes Gojo, Daniela Loetsch, et al. (Vienna)
Olivier Ayrault, Antoine Forget (Paris)
David Sumerauer (Prague)
Jan Koster (Amsterdam)
ITCC-P4 consortium

All people and patients who have contributed tissue samples for these studies!

dkfz.

UniversityHospital Heidelberg



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Acknowledgements:

Princess Máxima Center



Brain tumor organoids for
pediatric brain brain tumors