Inter and Intra-tumoral heterogeneity in pediatric sarcoma

"It is good to have hair-splitters & lumpers."
-Charles Darwin

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Rhabdomyosarcoma – “the most common soft tissue sarcoma of childhood”

Week

1	2	3	4	5	6	7	8	9	10	11	12	13	15
V	V	V	V	V	V	V	V	V	V	V	V	V	
A			A									A	Evaluation
C			C			C			C			C	
			Radiation Therapy →										

Week

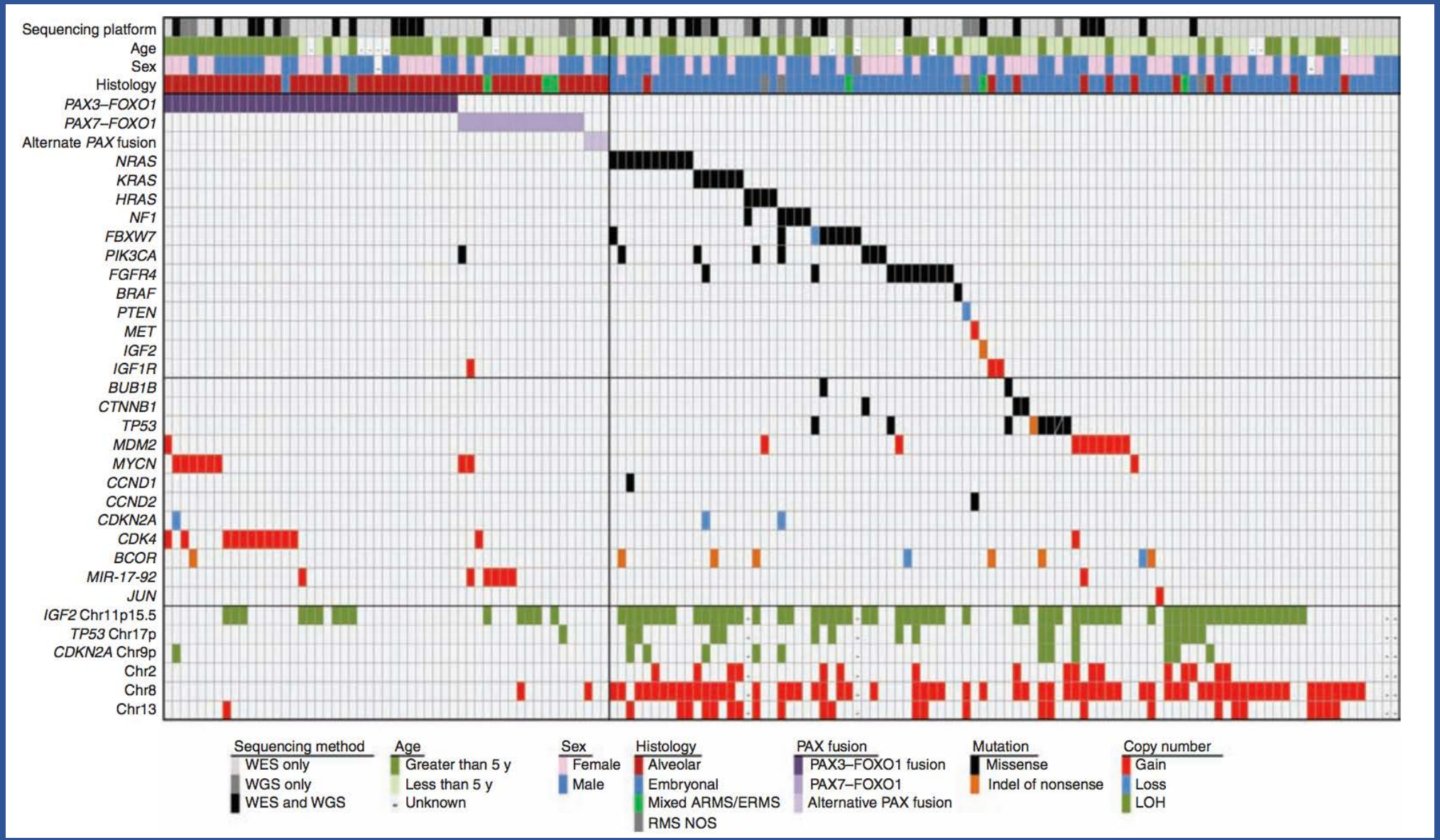
16	17	18	19	20	21	22	23	24	25	26	27	28	30
V			V	V	V	V	V	V	V			V	
A			A			A			A			A	Evaluation
C			C			C			C			C	

Week

31	32	33	34	35	36	37	38	39	40	41	42	43
V	V	V	V	V	V	V			V			
A			A			A			A			End of Therapy Evaluation
C			C			C			C			

	Drug	Age	Dose
V	VinCRIStine	< 1 year ≥ 1 year and < 3 years ≥ 3 years	0.025 mg/kg IV x 1 0.05 mg/kg IV x 1 (maximum dose 2 mg) 1.5 mg/m^2 IV x 1 (maximum dose 2 mg)
A	Dactinomycin	< 1 year ≥ 1 year	0.025 mg/kg IV x 1 0.045 mg/kg (maximum dose 2.5 mg) IV X 1
C	Cyclophosphamide	< 3 years ≥ 3 years	40 mg/kg IV X 1 1200 mg/m^2 IV X 1
Mesna and fluids will be used with Cyclophosphamide			
Neutrophil growth factor will be used in VAC and VC cycles. See Section 8 for specific directions.			
If there is an age change during treatment, use the new appropriate age dosing in the next cycle			

Rhabdomyosarcoma: Inter-tumor heterogeneity



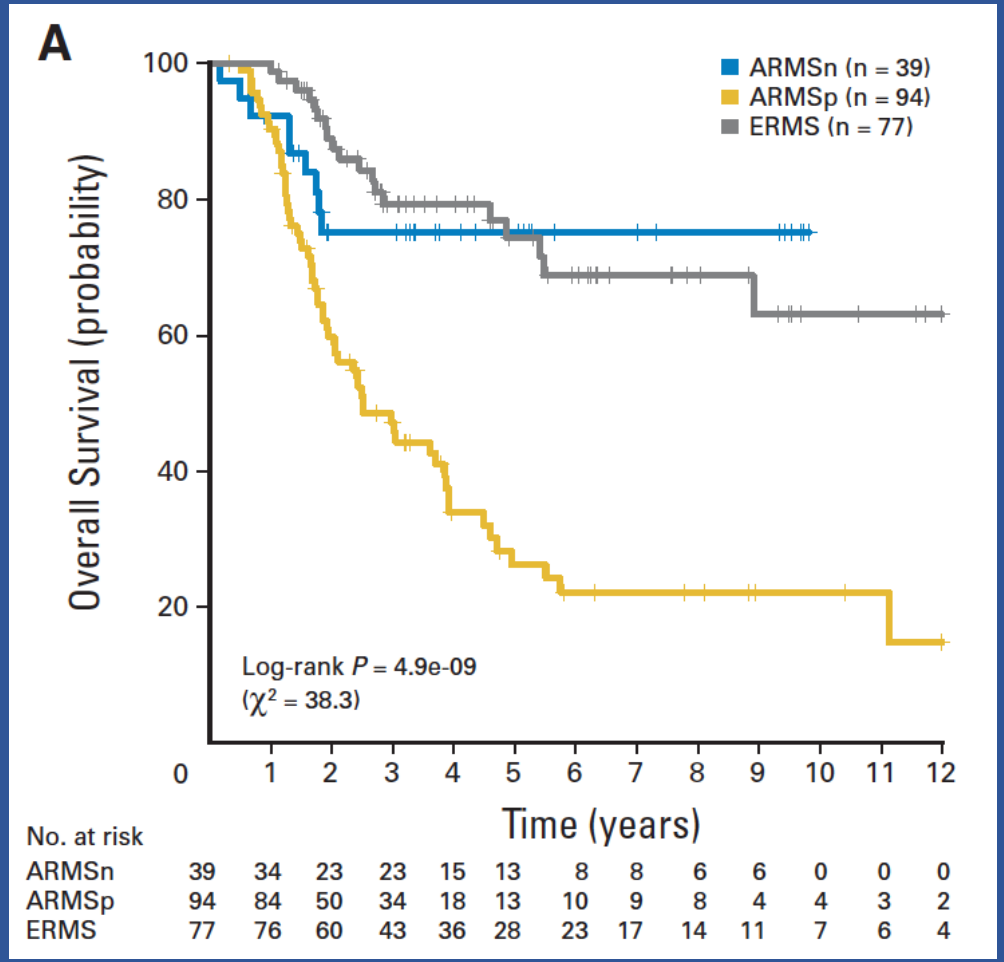
Shern et al. Cancer Discovery. 4: 216-231, 2014.

Can we use genetic information to further refine risk stratification?

Risk Group	Histology	Primary site	Initial resection	Distant metastases	Proportion of patients	EFS
Low	ERMS	Favorable	Any	None	32%	70-95%%
		Unfavorable	Yes	None		
Intermediate	ERMS	Unfavorable	No	None	27%	73%
	ARMS	Any	Any	None	25%	65%
High	ERMS	Any	Any	Present	8%	35%
	ARMS			Present	8%	15%

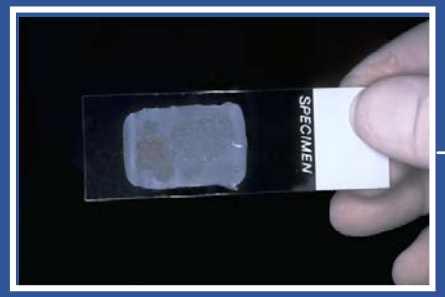
ERMS, embryonal rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma; EFS, event-free survival

Hawkins et al. Curr Opin Pediatr. 2014 Feb; 26(1): 50–56.



Williamson D et al. JCO 2010;28:2151-2158

ARST14B1 - Project Overview



2 unstained slides from Clinically annotated RMS cases

COG histology review and clinical annotation

NCI extraction and quantification of nucleotides and sample genotyping

DNA

Targeted Capture and Illumina sequencing

NCI Oncogenomics pipeline to call point mutations, indels, amplifications and deletions

RNA

RNAseq – Shern

Nanostring assay

Mike Arnold

Nationwide Childrens

Correlation between the observed genetic alterations (RAS pathway alterations vs no RAS pathway alteration); expression signatures and clinical outcome

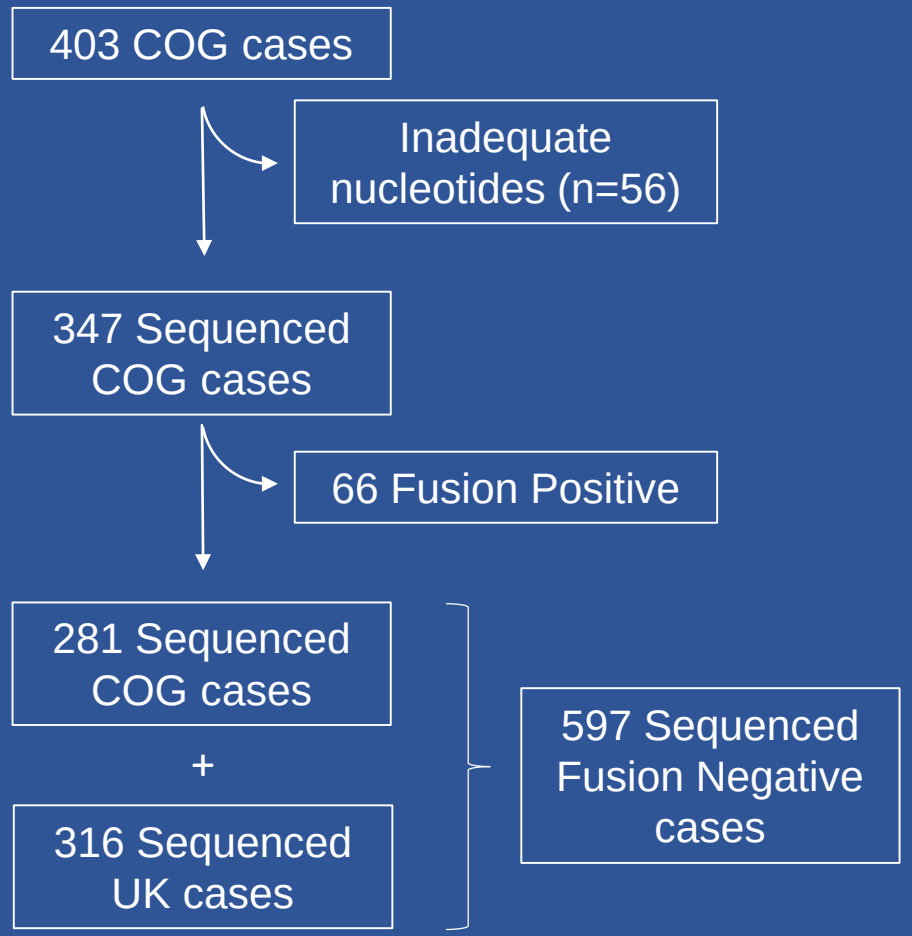
NATIONAL CANCER INSTITUTE
Center for Cancer Research

COG ARST14B1 Summary

AKT1	*CDK4*	*GAB1*	*MYCN*	*PTEN*
ALK	*CDKN2A*	*HRAS*	*MYOD1*	*PTPN11*
ARID1A	*CTNNB1*	*IGF1R*	*NF1*	*ROBO1*
ATM	*DICER1*	*IGF2*	*NRAS*	*SMARCA4*
BCOR	*ERBB2*	*KRAS*	*PDGFRA*	*SOS1*
BRAF	*FBXW7*	*MDM2*	*PIK3CA*	*SOS2*
CCND1	*FGFR1*	*MET*	*PKN1*	*TP53*
CCND2	*FGFR4*	*MTOR*	*PTCH1*	

Tier I Calls - (Somatic/germline status unknown)

- Hot Spot or stop or deleterious indel
- High copy number amplification
- Deep deletion of any included gene



Clinical Characteristics of the COG cohort

Characteristic	n=347 (%)
Sex	
Male	234 (67)
Female	113 (33)
Age at presentation (years)	
Median	6.4
Range	0.02-37.8
Tumor Histology	
Alveolar	66 (19)
Embryonal	219 (63)
Embryonal with diffuse anaplasia	15 (4)
Embryonal with focal anaplasia	6 (2)
Mixed Alveolar and Embryonal	2 (<1)
Mixed Embryonal and Spindle cell	9 (3)
Spindle cell	16 (5)
Botryoid	10 (3)
Rhabdomyosarcoma NOS	3 (1)
Cytology Specimen	1 (<1)
Anatomic Group	
Bladder Prostate Group 3	18 (5)
Bladder Prostate Group 4	6 (2)
Extremity Group 3	16 (5)
Extremity Group 4	35 (10)
Female GU	8 (2)
Head and Neck	29 (8)
Orbital	25 (7)
Parameningeal Group 3	46 (13)
Parameningeal Group 4	14 (4)
Paratesticular	64 (19)
Pilot Study No Data	10 (3)
Retroperitineum/Peritineum/Trunk Group 3	44 (13)
Retroperitineum/Peritineum/Trunk Group 4	30 (9)
Risk Group	
Low	93 (27)
Intermediate	131 (38)
High	123 (35)
Variant Calls	
Median	1
Range	0-5

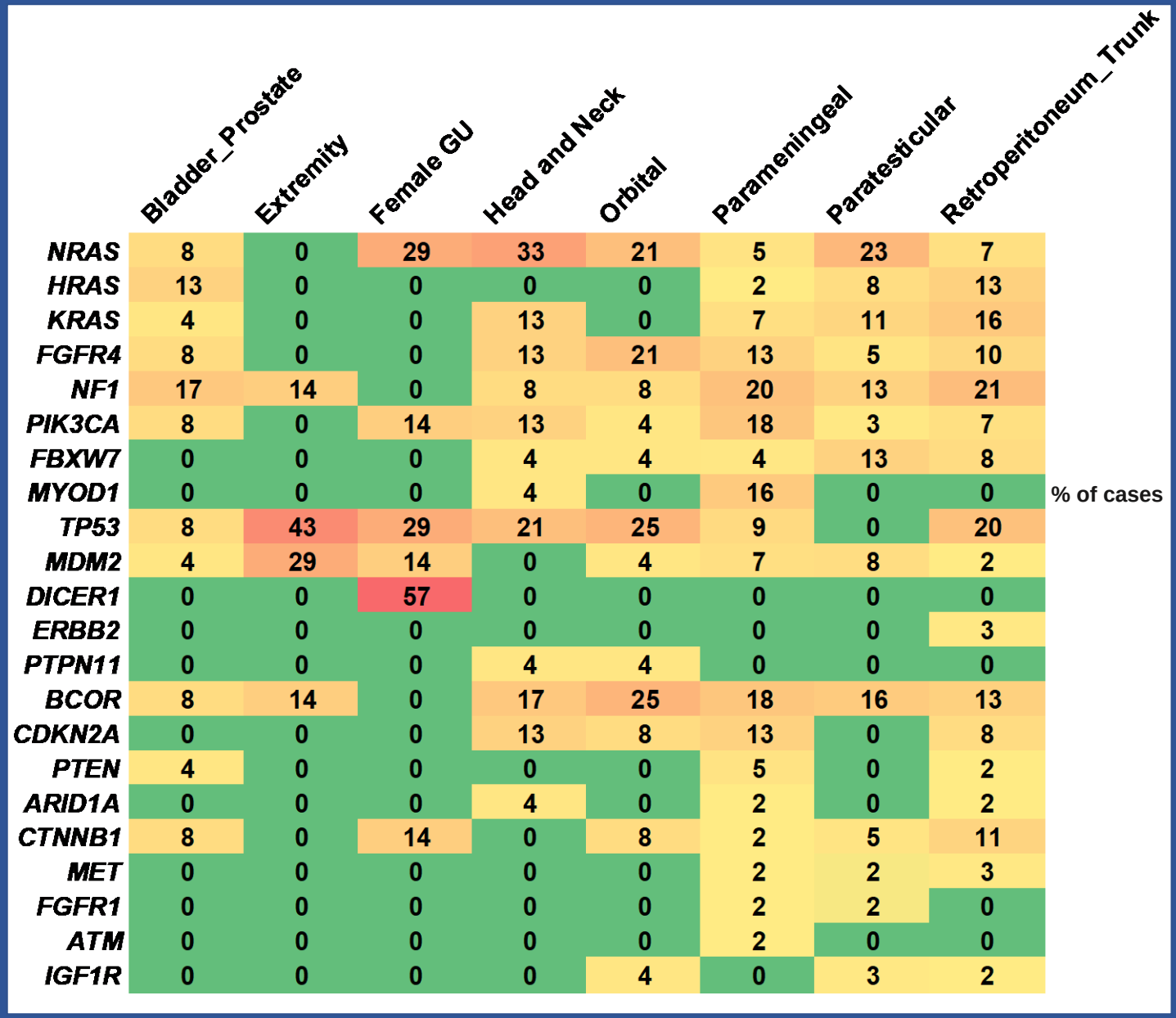
Mutation Summary

	Fusion Negative Cases (n=)	Total Fusion Negative Cases	Fusion Negative Cases (%)	Fusion Postive Cases (n=)	Total Fusion Positive Cases	Fusion Positive Cases (%)
BCOR	43	281	15%	3	66	5%
NF1	42	281	15%	1	66	2%
NRAS	41	281	15%	0	66	0%
TP53	36	281	13%	3	66	5%
FGFR4	26	281	9%	0	66	0%
KRAS	25	281	9%	0	66	0%
PIK3CA	23	281	8%	2	66	3%
HRAS	18	281	6%	1	66	2%
FBXW7	17	281	6%	0	66	0%
CDKN2A	17	281	6%	0	66	0%
MDM2	16	281	6%	1	66	2%
CTNNB1	16	281	6%	0	66	0%
MYOD1	11	281	4%	0	66	0%
PTEN	5	281	2%	0	66	0%
DICER1	4	281	1%	0	66	0%
MET	4	281	1%	0	66	0%
IGF1R	4	281	1%	1	66	2%
ARID1A	3	281	1%	0	66	0%
ERBB2	2	281	1%	0	66	0%
PTPN11	2	281	1%	0	66	0%
FGFR1	2	281	1%	0	66	0%
PTCH1	1	281	0%	0	66	0%
ATM	1	281	0%	0	66	0%
CDK4	1	281	0%	14	66	21%
MYCN	0	281	0%	9	66	14%

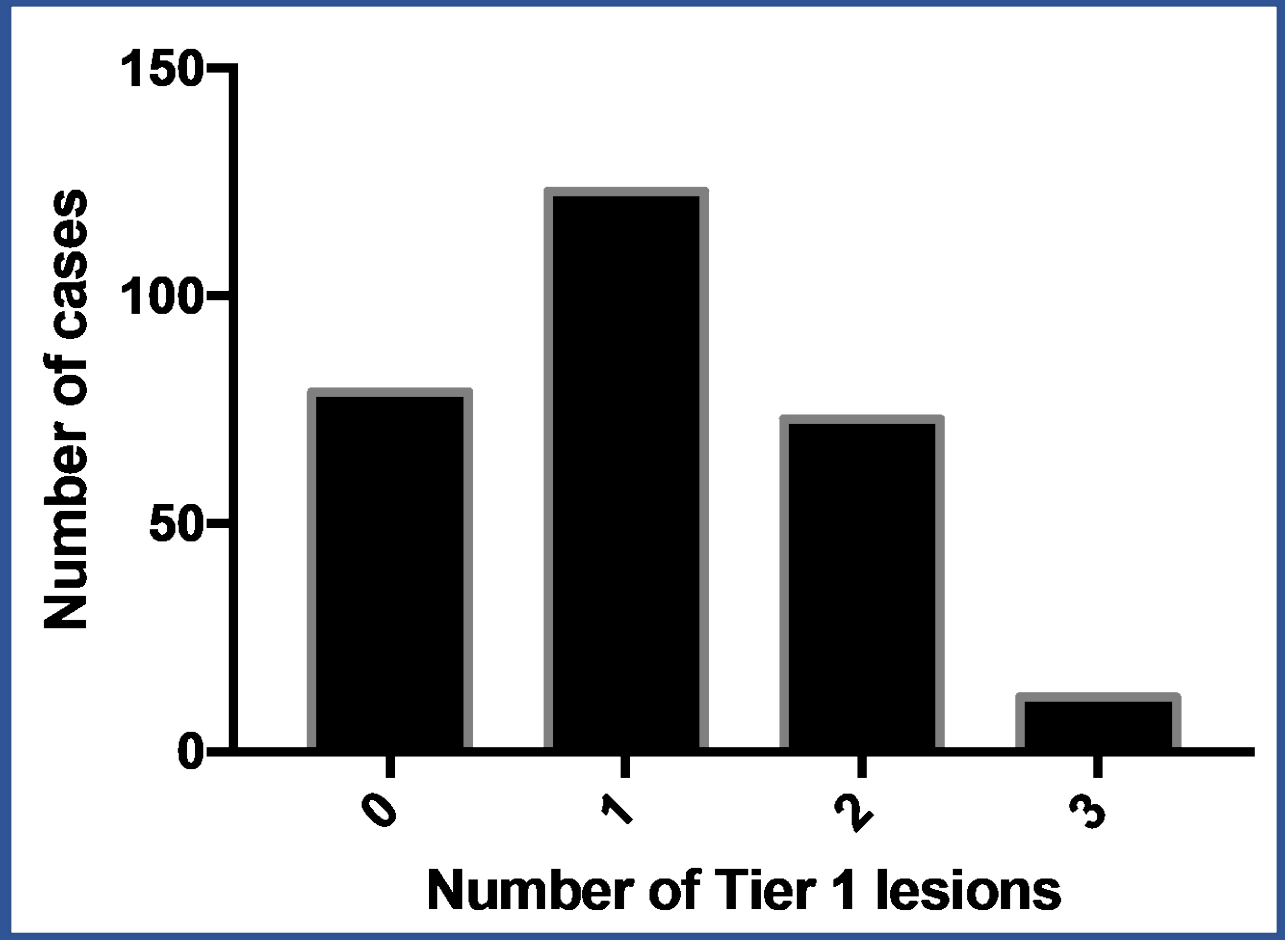
Identified at least one Tier 1 driver in 221/281 (80%) fusion negative cases

Percentage of cases summarized by anatomy

- *TP53* pathway mutations are common in fusion negative extremity lesions
- Female genitourinary cases account for all of the *DICER1* lesions
- *HRAS* and *KRAS* do not occur in orbital tumors
- *MYOD1* mutations are restricted to the head



1 tumor ≠1 genetic lesion



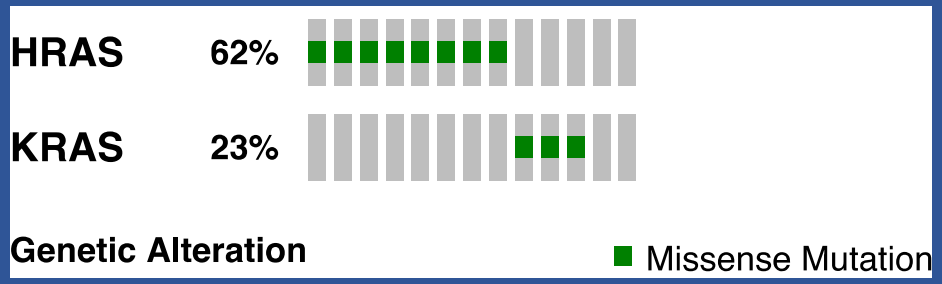
Hypothesis: Fusion Negative Rhabdomyosarcoma is polyclonal?

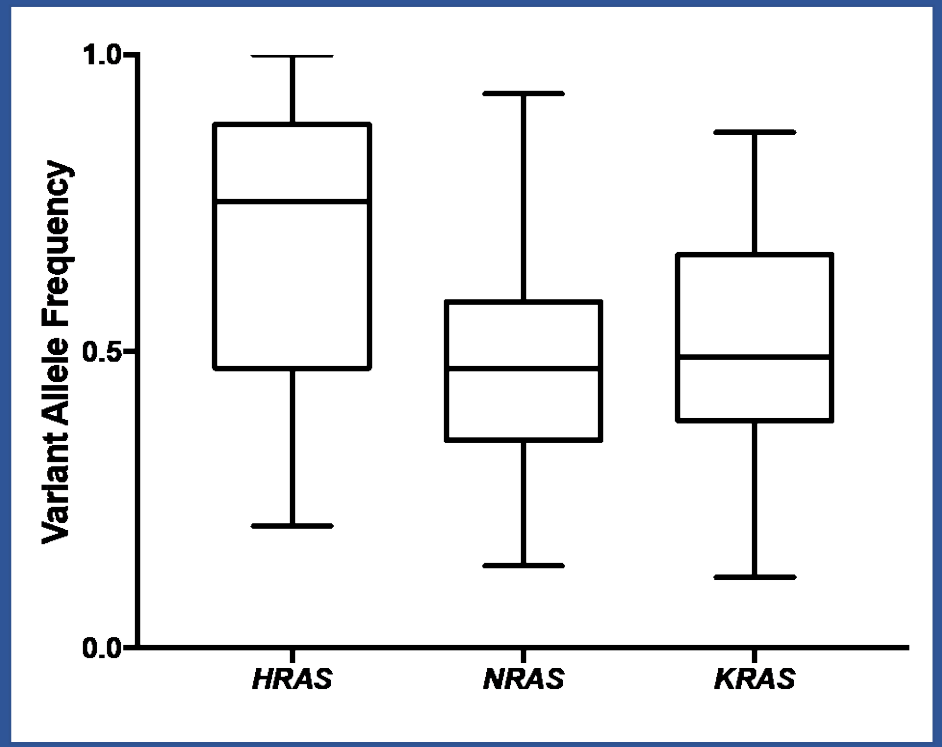
Hypothesis: Increased number of mutations leads to a worse outcome

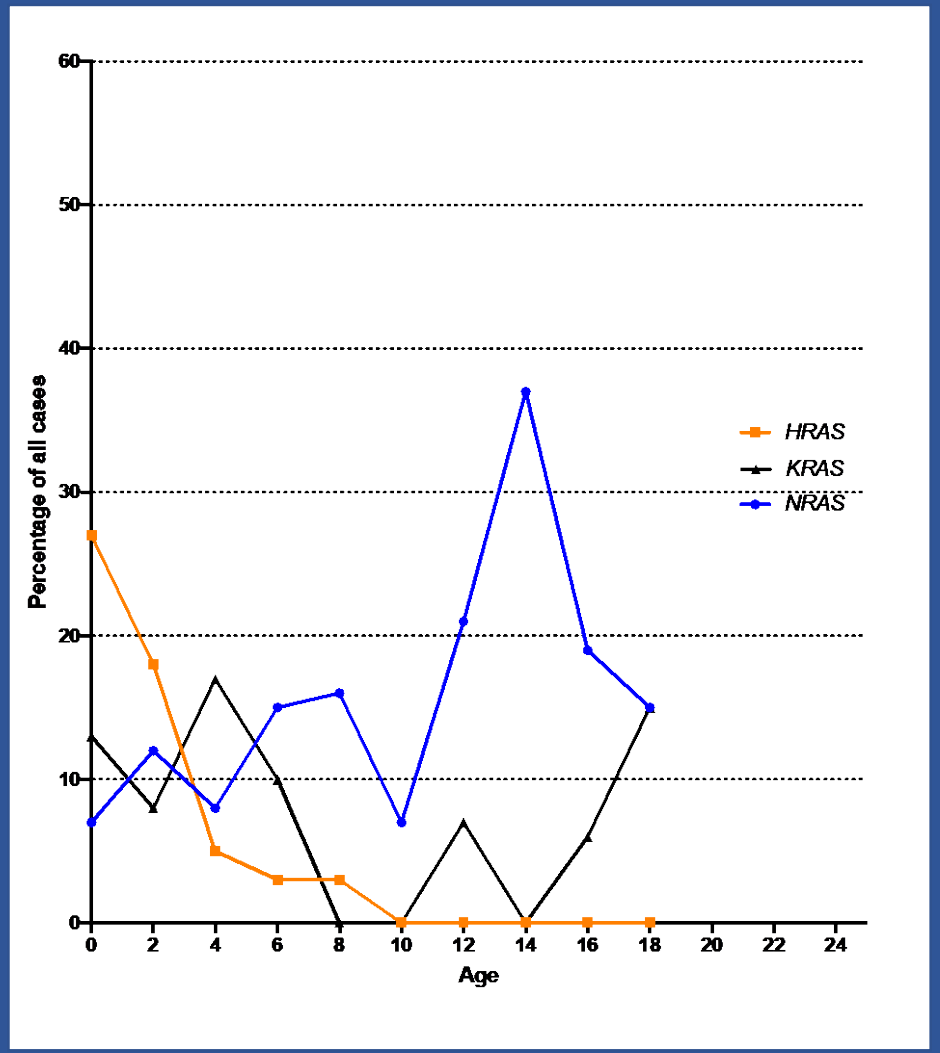
What genes go together?

	Total Cases (n=)	Co existing lesion (n=)	Co-Existing lesion (%)	No co-existing lesion (n=)	No co-existing lesion (%)
HRAS	18	7	39%	11	61%
KRAS	25	10	40%	15	60%
MDM2	16	7	44%	9	56%
DICER1	4	2	50%	2	50%
MET	4	2	50%	2	50%
NRAS	41	23	56%	18	44%
CTNNB1	16	10	63%	6	38%
TP53	36	24	67%	12	33%
FGFR4	26	18	69%	8	31%
BCOR	43	33	77%	10	23%
NF1	42	33	79%	9	21%
PIK3CA	23	19	83%	4	17%
FBXW7	17	15	88%	2	12%
CDKN2A	17	15	88%	2	12%
MYOD1	11	10	91%	1	9%
IGF1R	4	4	100%	0	0%

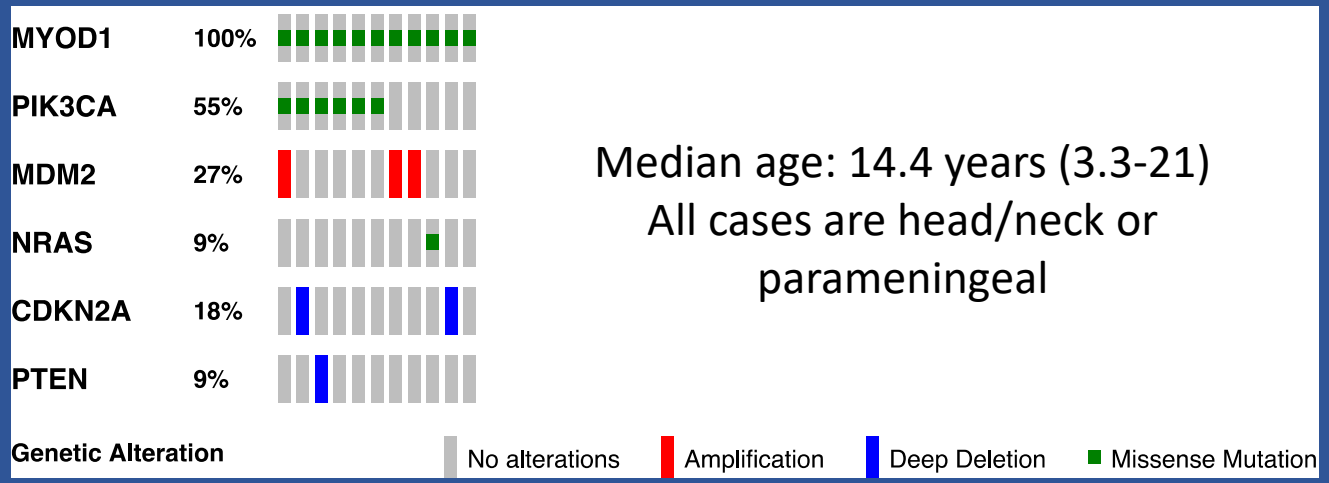
Infants less than 1 year old and the distribution of RAS isoform mutations by age



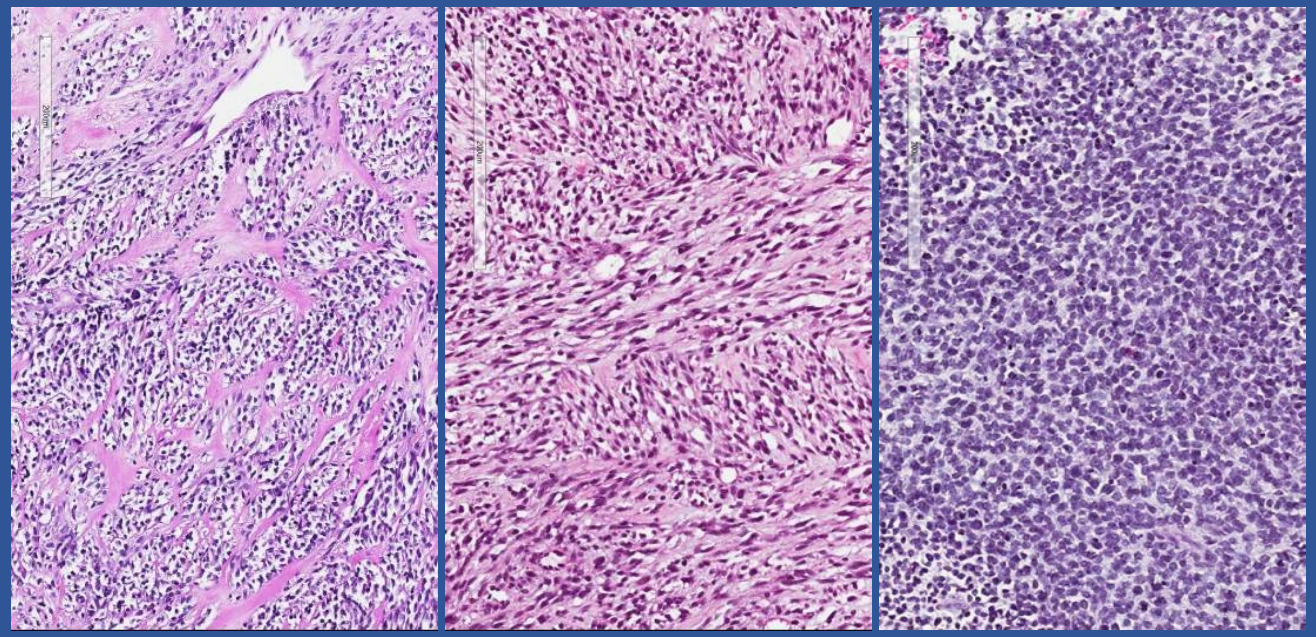


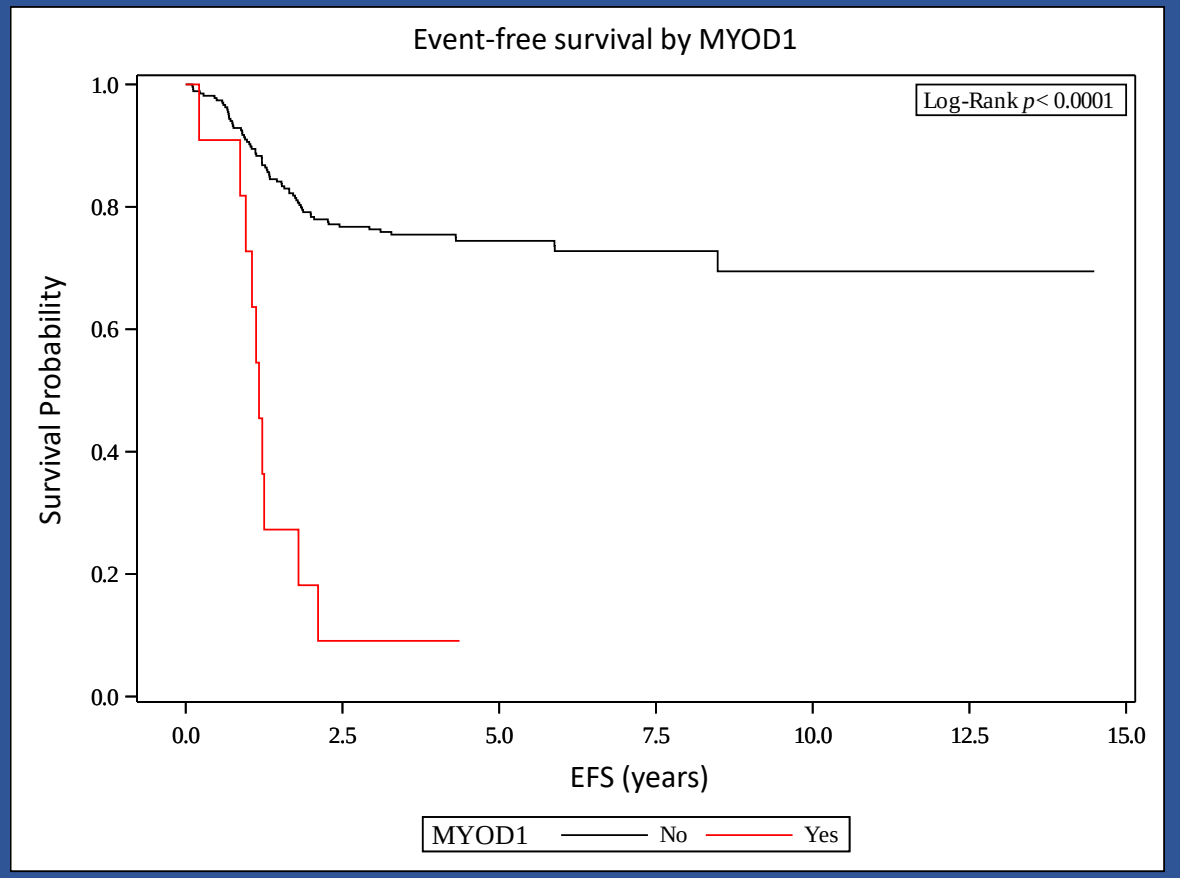


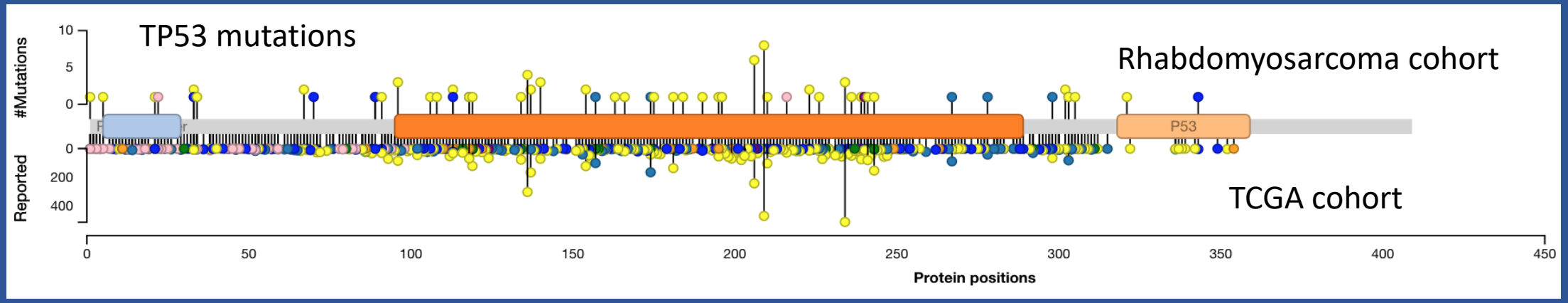
MYOD1 mutations are associated with a dismal outcome

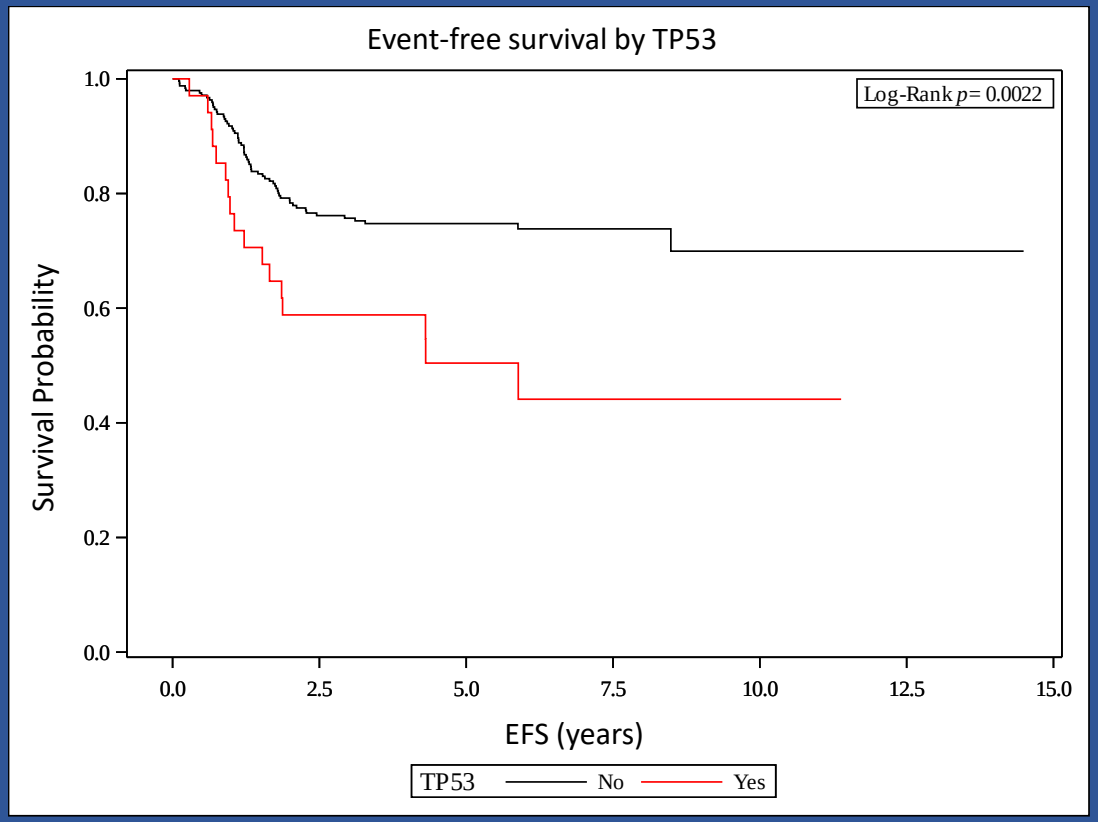


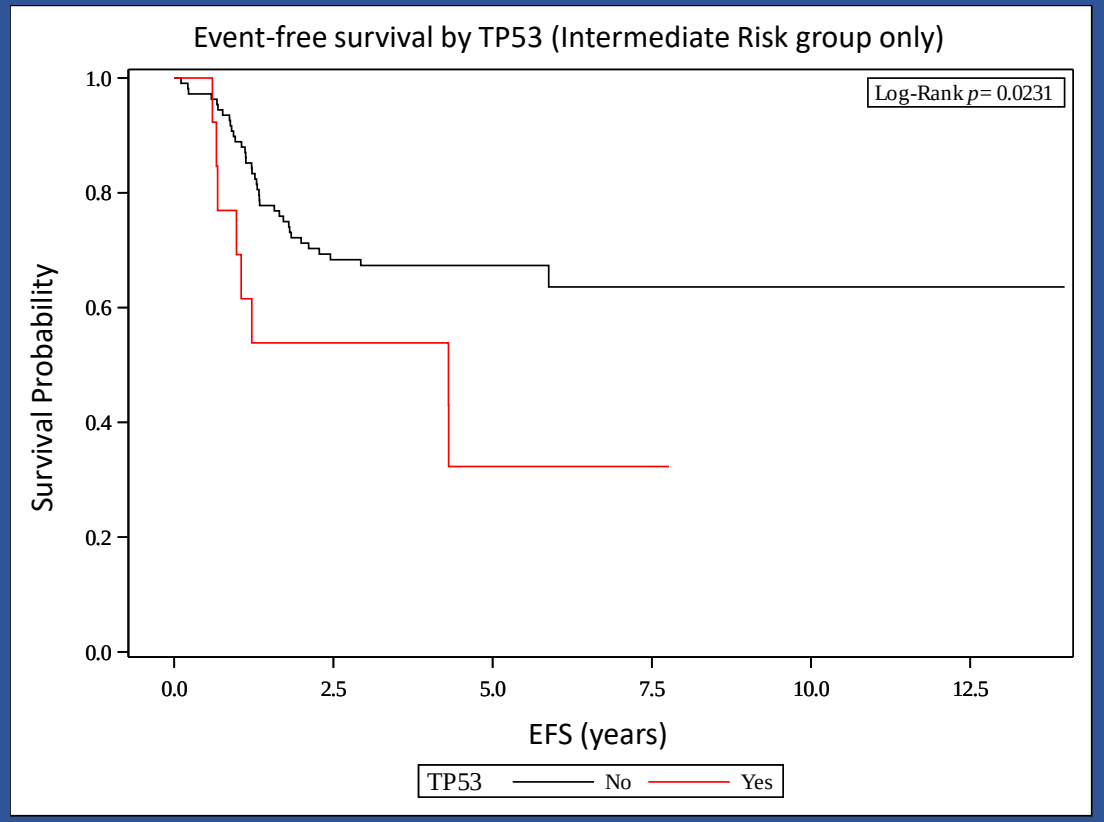
Median age: 14.4 years (3.3-21)
All cases are head/neck or parameningeal



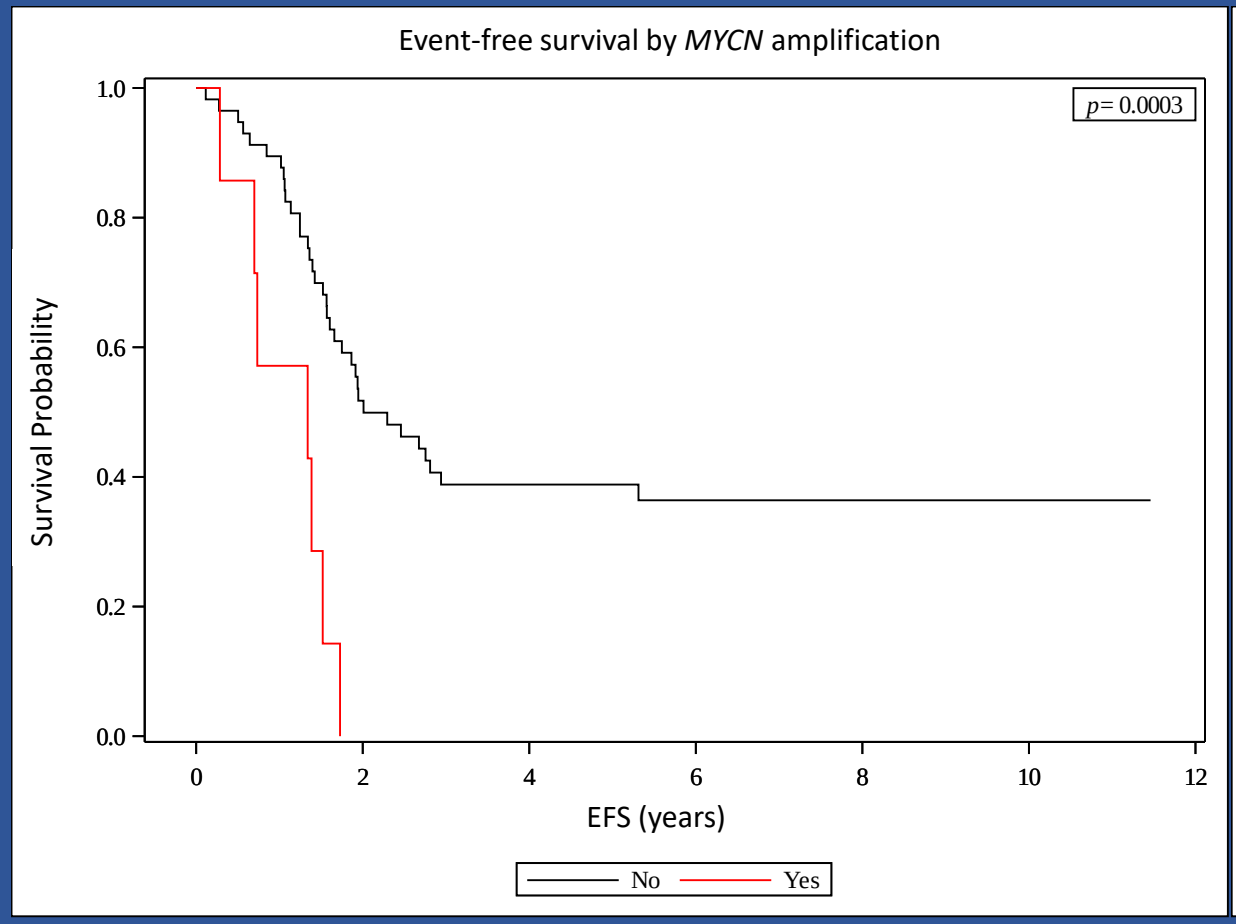


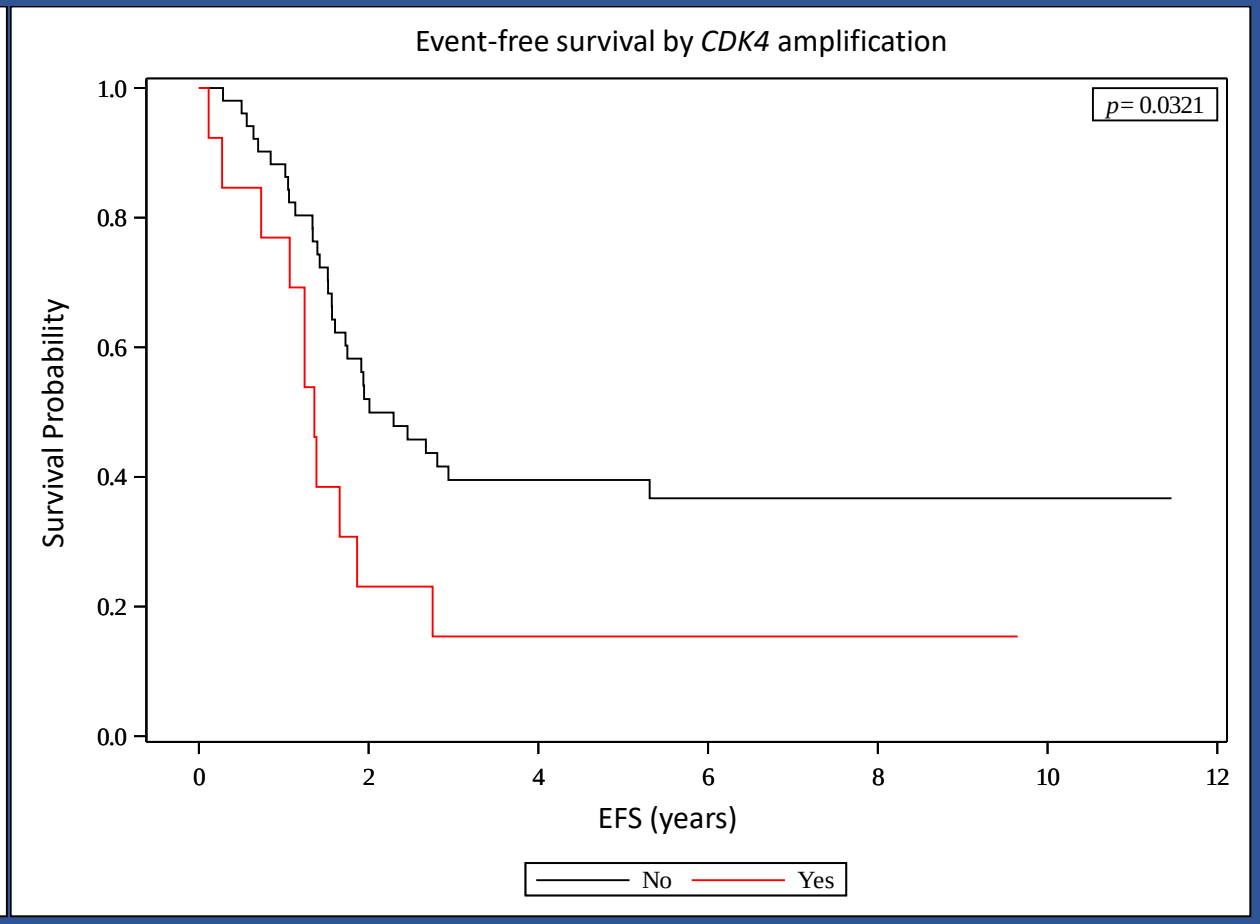

TP53 mutations
Rhabdomyosarcoma cohort
TCGA cohort
#Mutations
Reported
P53
Protein positions


Event-free survival by TP53
Log-Rank p= 0.0022
Survival Probability
EFS (years)
TP53 No Yes

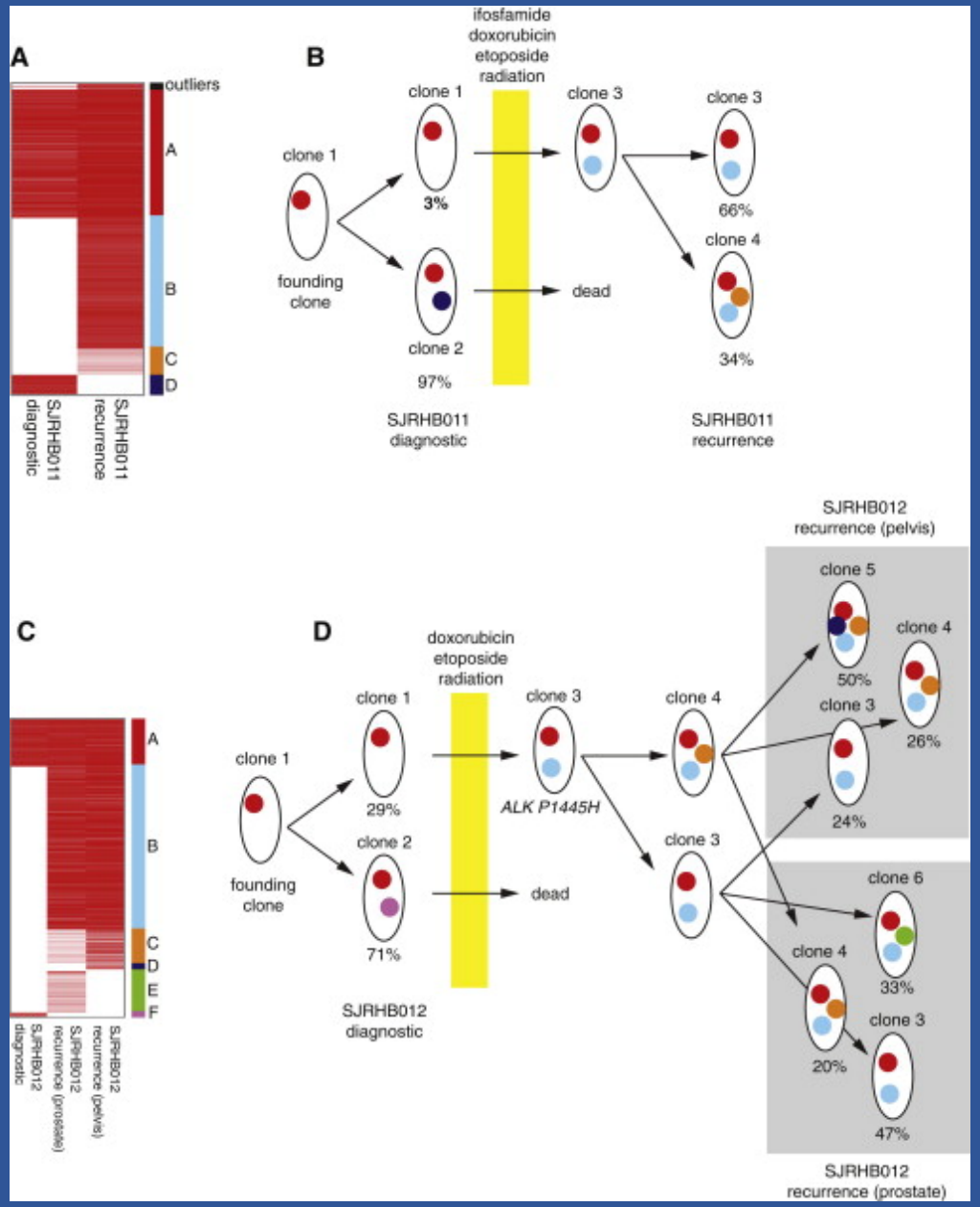

Event-free survival by TP53 (Intermediate Risk group only)
Log-Rank p= 0.0231
Survival Probability
EFS (years)
TP53 No Yes

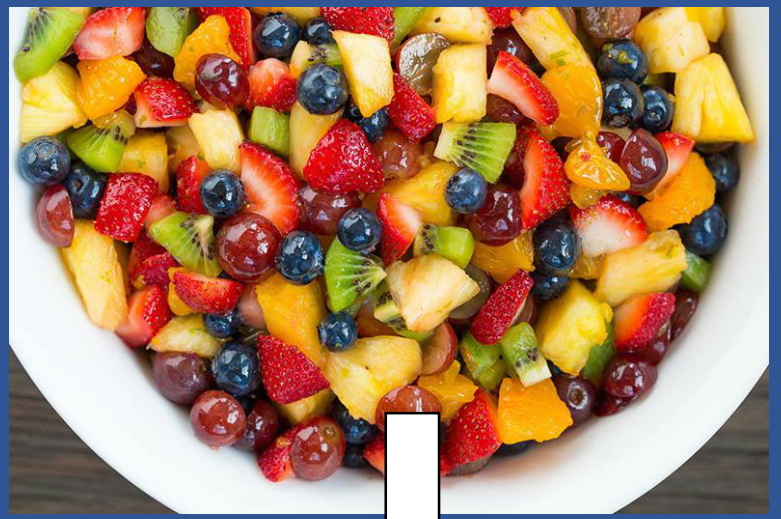
MYCN and *CDK4* amplifications are poor prognostic modifiers in PAX fusion positive tumors





Pediatric Sarcoma: Intra-tumor Heterogeneity

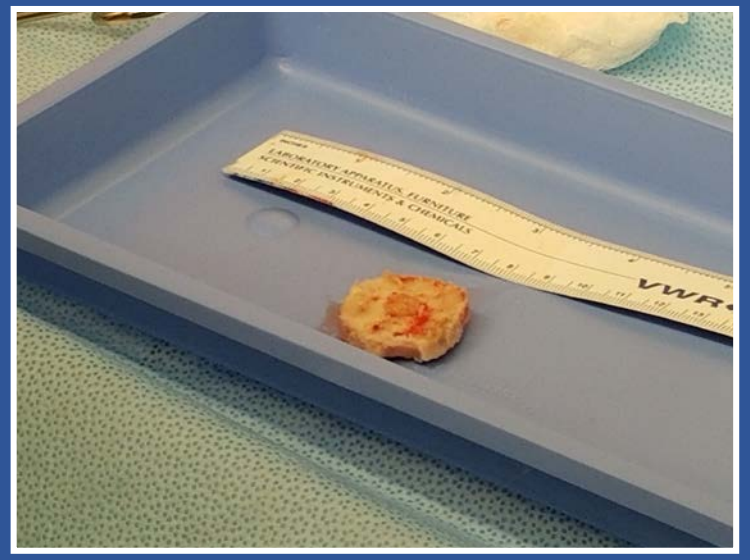


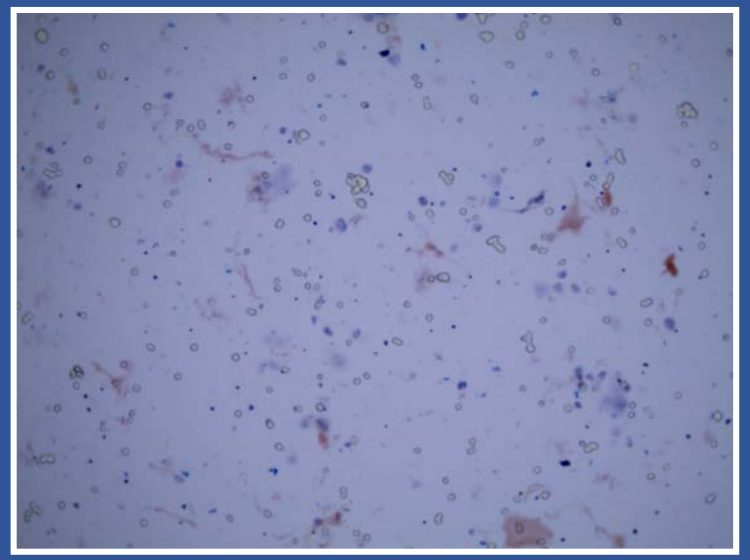


Single cell sequencing

Chen et al. Cancer Cell 24, 710-724, 2013

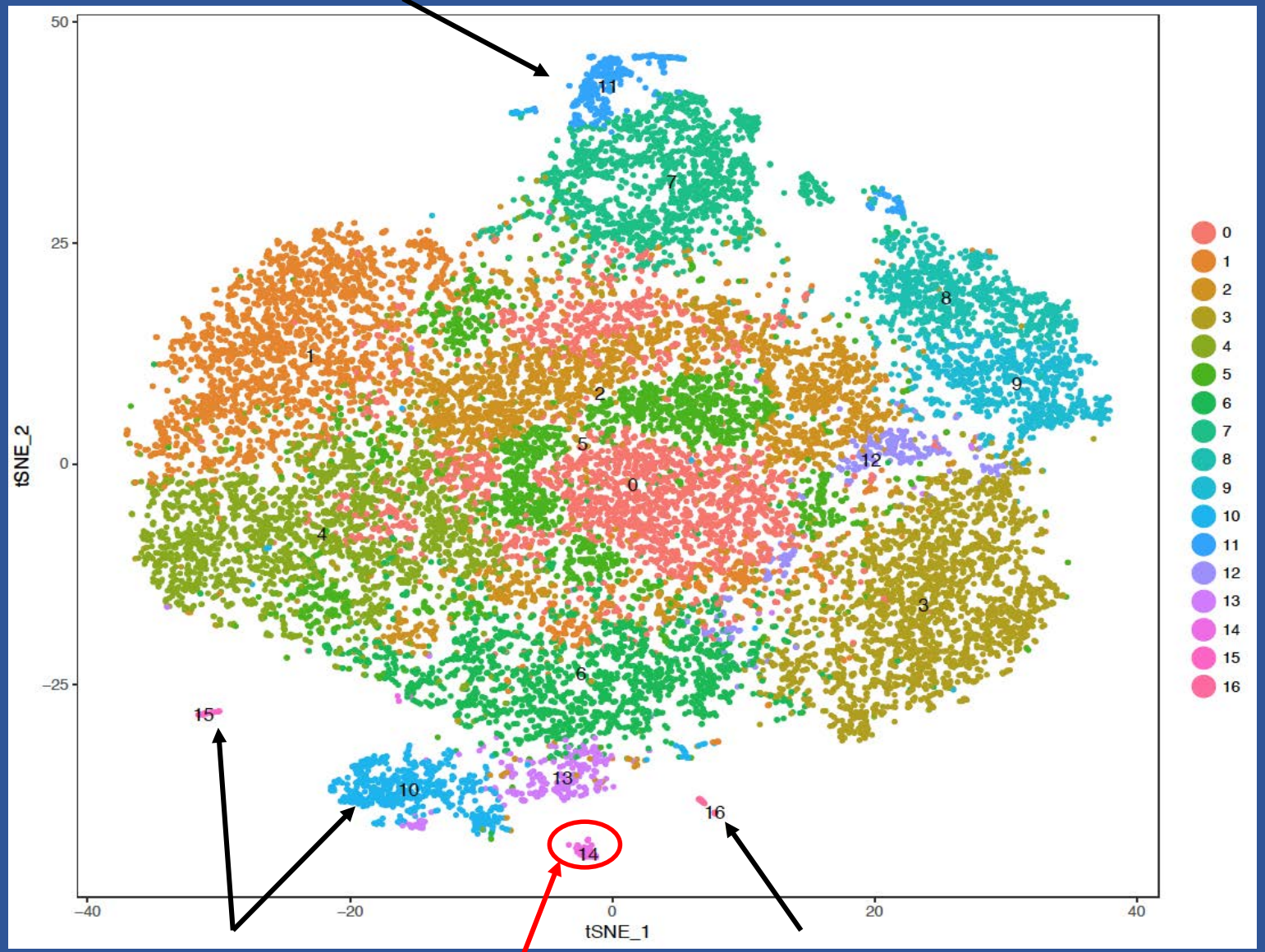
Skull based metastatic tumor





Carly Sayers
Xiyuan Zhang

G2/M – TOP2A, FOXM1, Kinesins, Centrosomal genes

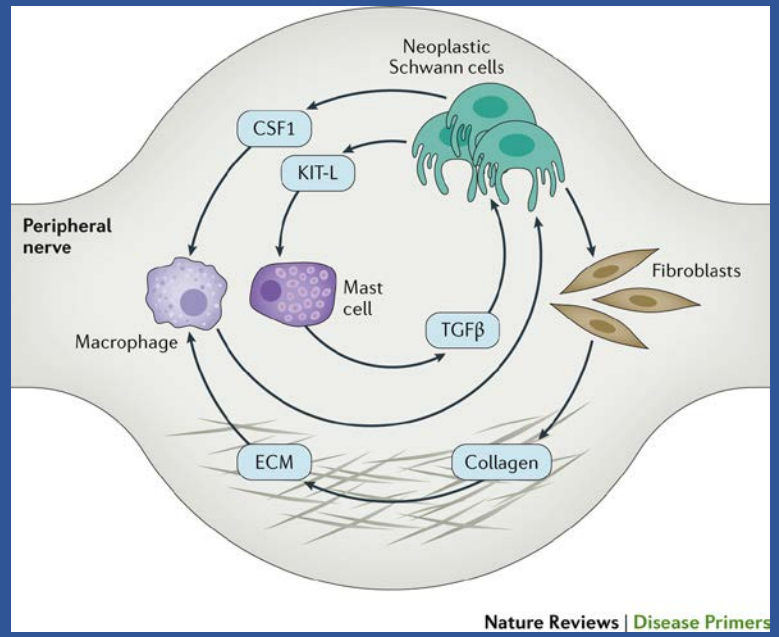


Collagens, Fibrillin, IGFBP's

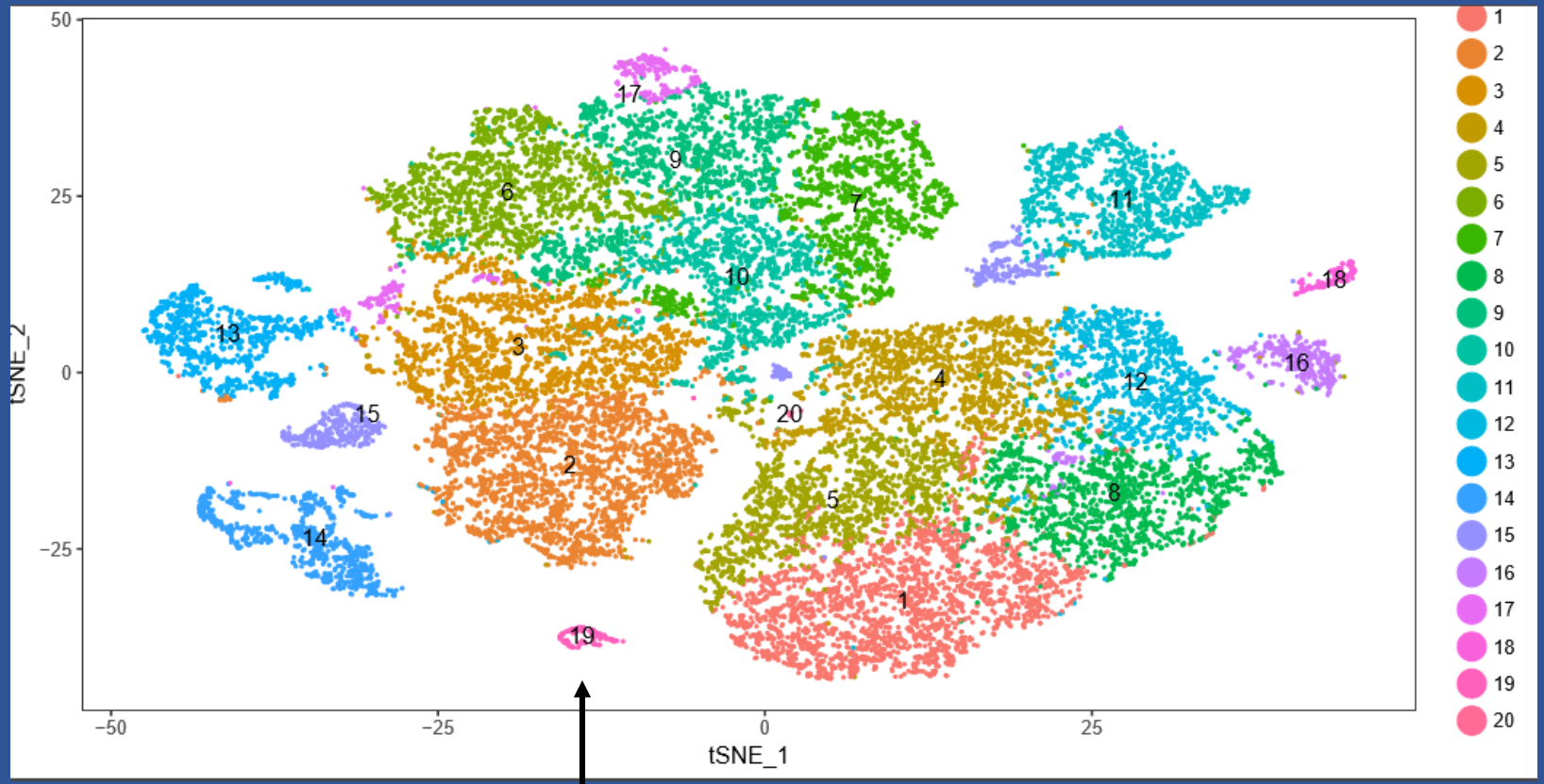
BIRC3, KLF10

CD4+, CD37+, CD53+, CD74+ Producing Complement and Lysozyme

Atypical Neurofibroma with concern for Malignant Peripheral Nerve Sheath Tumor

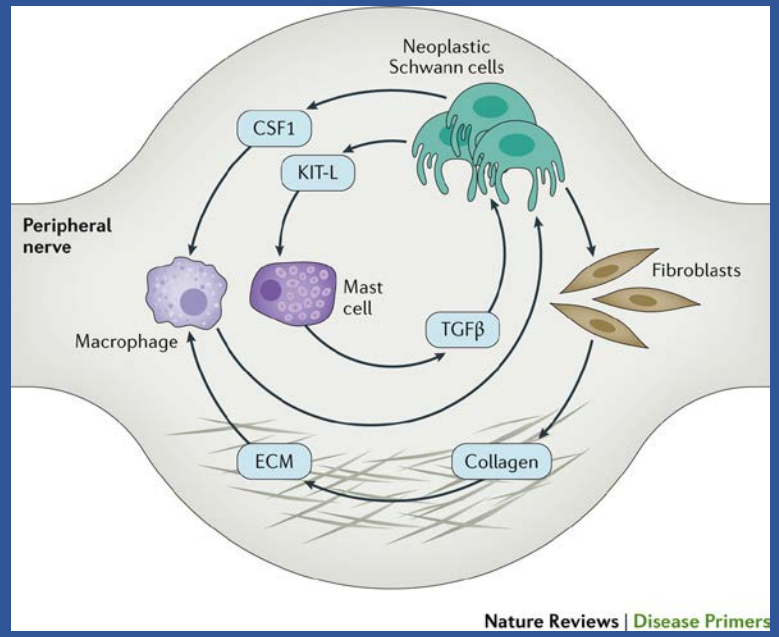


Gutmann, D. H. *et al.* (2017)

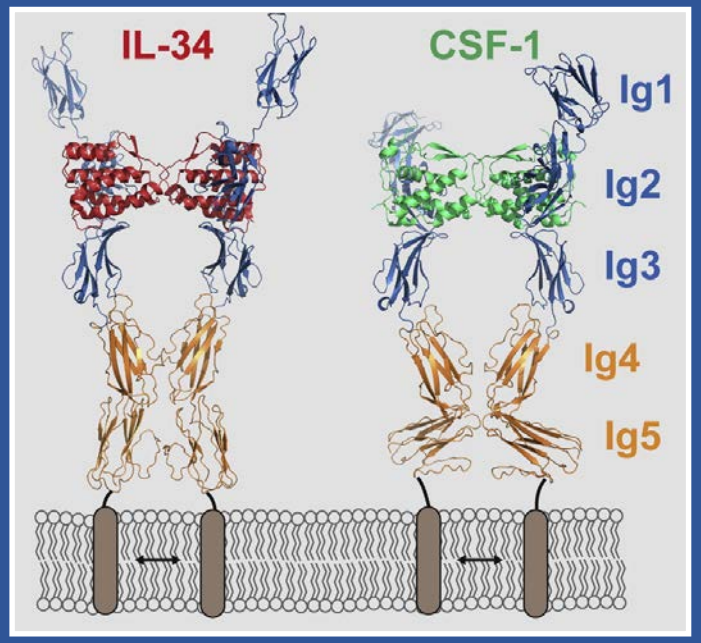


Neoplastic Schwann cells:
NRXN1, *SOX10*, *S100B*

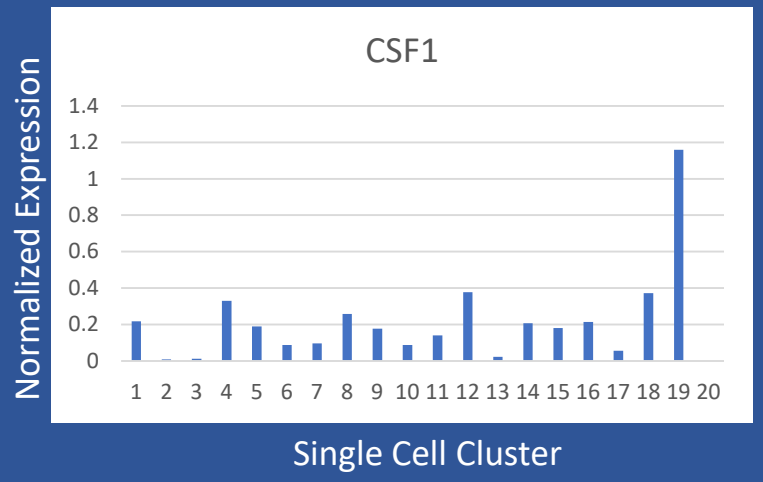
Single cell sequencing identifies IL34 as a novel therapeutic target in NF1 tumors

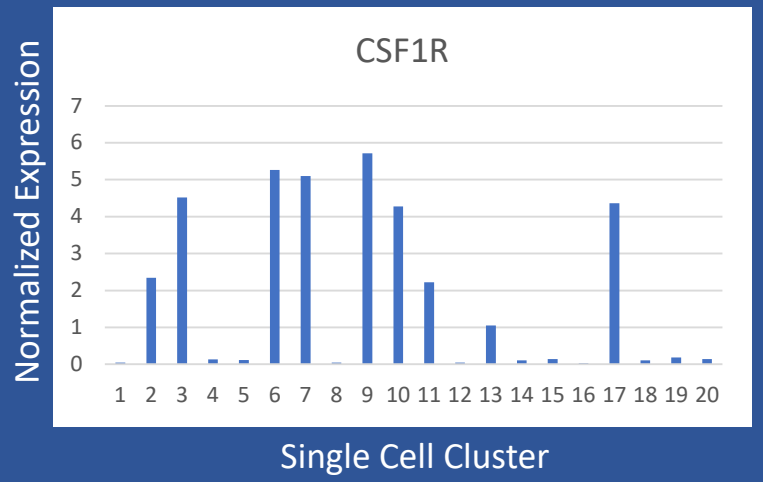


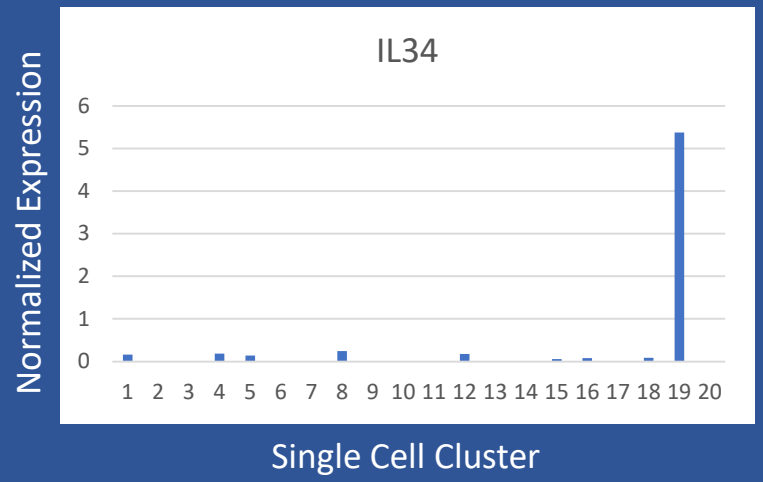
Gutmann, D. H. *Nature Reviews*. (2017)



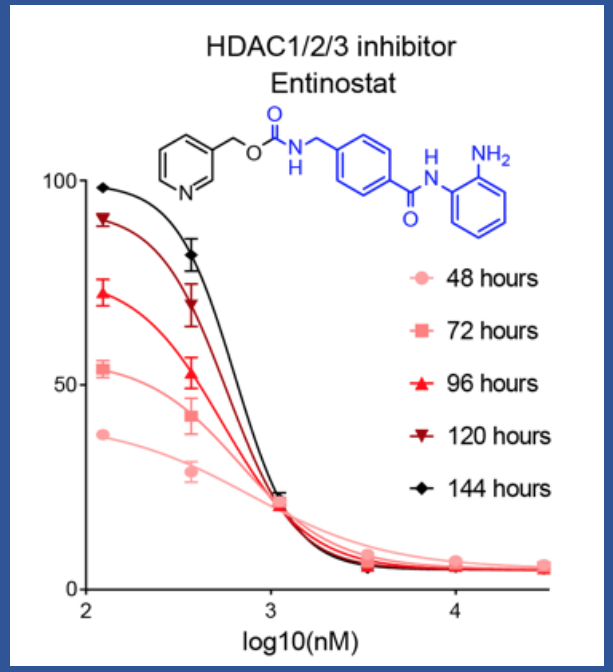
Felix, J. *et al. Structure* (2013)

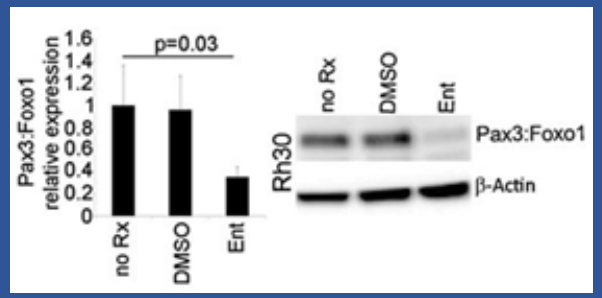




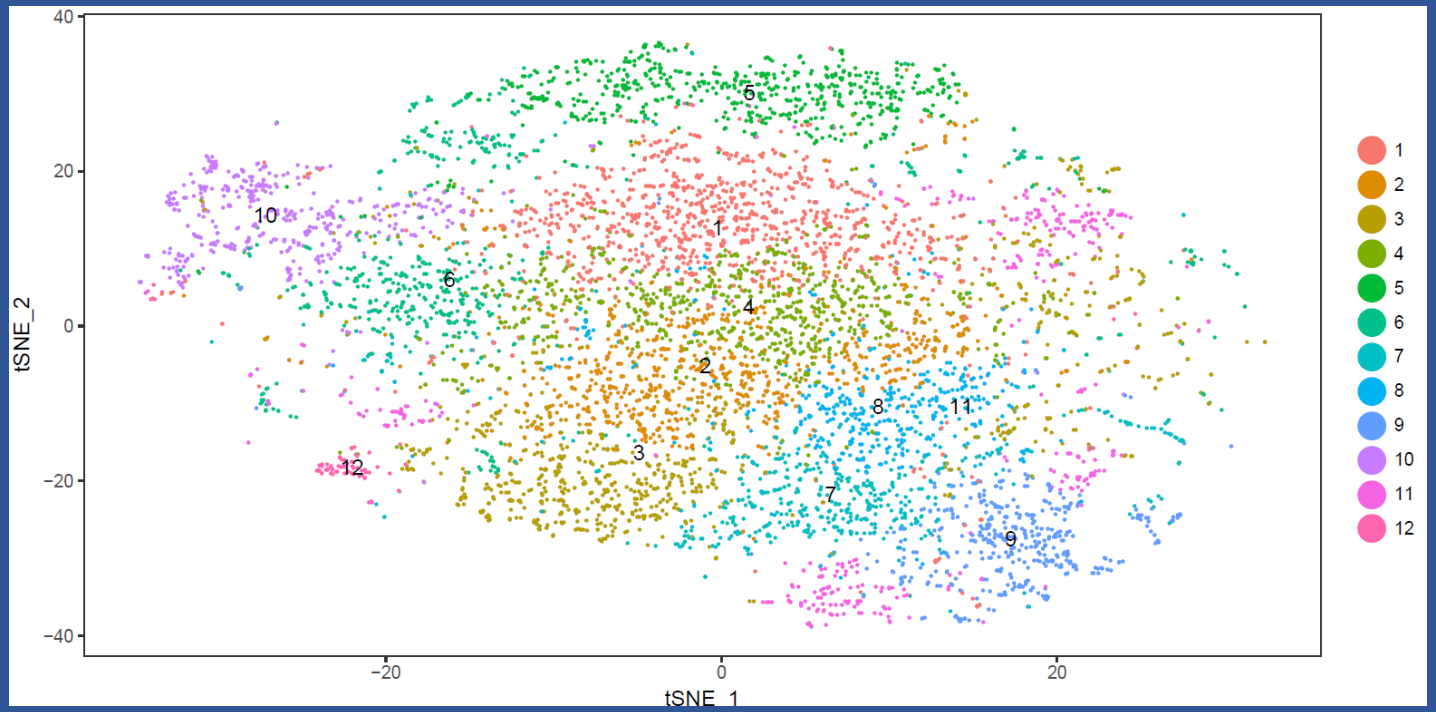


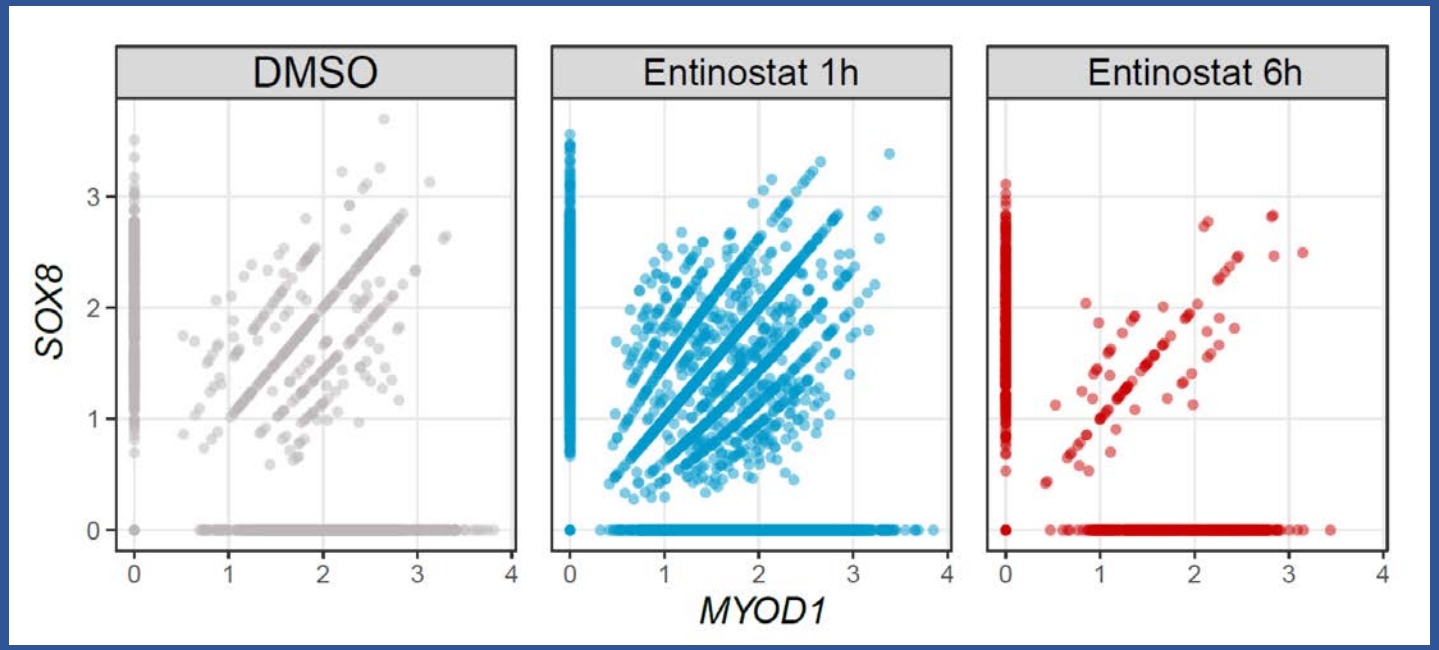
HDAC inhibitors are potent repressors of PAX3-FOXO1 transcriptional activity.





Abraham, J. *et al. Genes Dev* (2014)

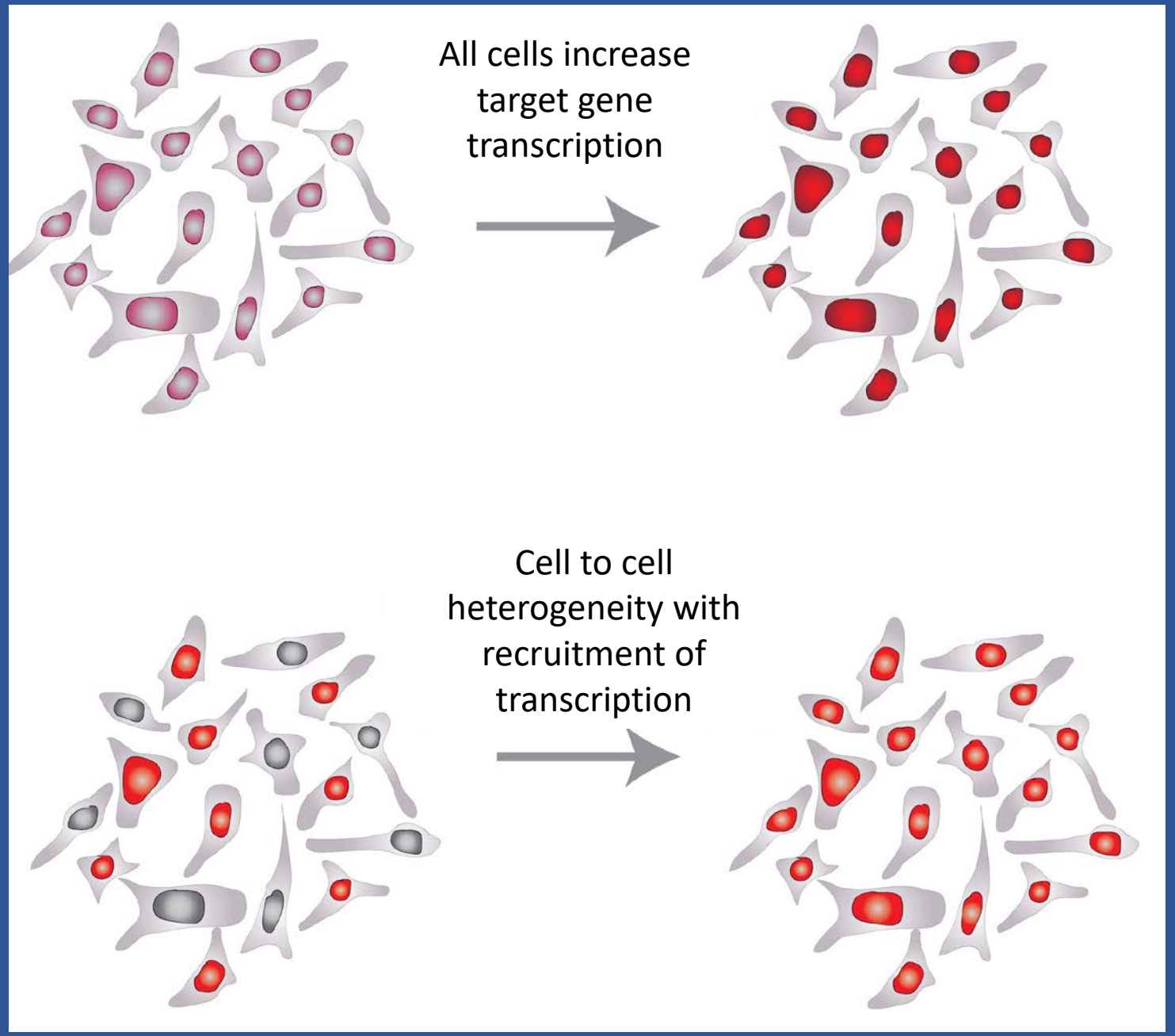




Even in a “homogenous” cell culture, single cell RNAseq shows cell to cell variability

Bulk sequencing averages across the population thereby losing information about rare cell populations

Definition of these cell populations has major implications for our understanding of tumor evolution and therapeutic resistance



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Kristie Jones

Belynda Hicks

NCI CCR

Javed Khan

Berkley Gryder

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Ashley Walton

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CHILDREN'S ONCOLOGY GROUP

St. Baldrick's FOUNDATION Conquer Childhood Cancers

The Lasker Clinical Research Scholars Program at the NIH

Goal – To grow the diminishing pool of talented clinical/translational researchers.

Total program duration 8 years:

- Years 1-5: NIH Intramural Research Program full support (salary and research support)
- Years 6-8: salary and/or research support of up to $500,000/year at outside institution, OR continuation in Intramural Research Program

Candidates – Early stage clinical researchers, within 10 years of completing core residency, with the ability to conduct independent research.

http://www.nih.gov/science/laskerscholar/

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