

ITCC EDUCATION MEETING UTRECHT

Workshop 5 – Simulated Molecular Tumor Board

Example 3

Neuroblastoma

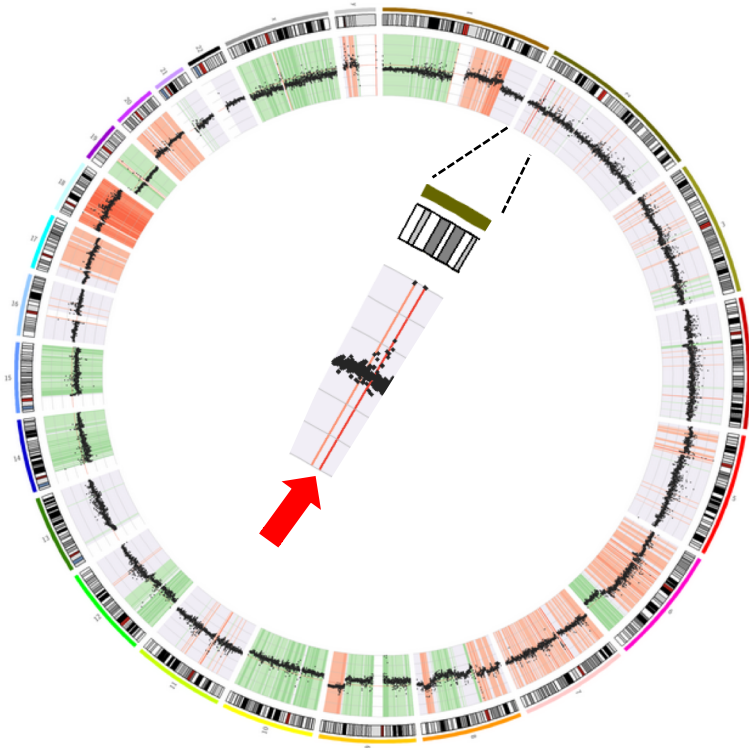
Oncologist: Clinical synopsis

- Gender: Male
- Age: 5 years
- Primary Tumor:
 - Neuroblastoma stage IV, bone & marrow metastases
 - positive for MYCN amplification: high-risk stratified
- History:
 - high risk protocol, resection
 - new lesion tibia, chemotherapy
 - thereafter progressive disease (sacral, tibiae, abdomen)

Pathologist: Tumor biopsy

- Sample: progressive disease
- Type: tru cut tibia lesion
- Quality: good
- Tumor content: 40% (=minimum allowed to enter sequencing study)
- DNA isolation: good
- RNA isolation: RIN 6.6 on scale from 1 (degraded) to 10 (excellent)
- Germline DNA: blood sample

Genomics: Copy number profile



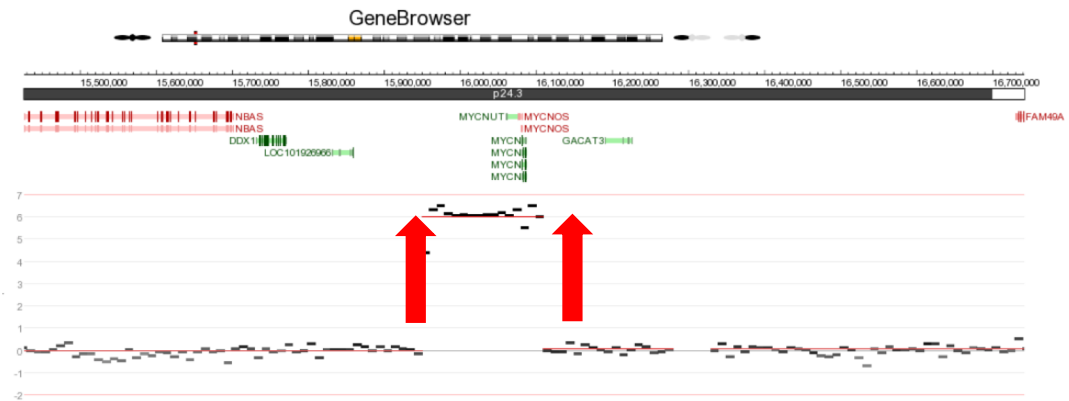
circos plot with tracks for

- chromosome and band
- copy number log2 ratio for tumor vs. normal (0 = diploid)

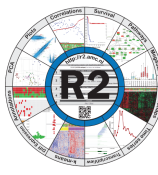
▼ CopyNumber list

gains (red)				losses (green)			
link	Gene	logfold	Info	link	Gene	logfold	Info
View	MYCN	6.0239	chr2:15950000-16109999 (159999)	View	SPRED1	-0.6854	chr15:38510000-38789999 (279999)
View	BCL2	0.9953	chr18:59860000-60859999 (999999)	View	PRKDC	-0.6275	chr8:48210000-49209999 (999999)
View	PHLPP1	0.9953	chr18:59860000-60859999 (999999)	View	BRDT	-0.6249	chr1:92440000-93439999 (999999)
View	YES1	0.8987	chr18:10000-1009999 (999999)	View	JAK2	-0.6091	chr9:4970000-5969999 (999999)
View	SS18	0.7669	chr18:23580000-24009999 (429999)	View	CD274	-0.6091	chr9:4970000-5969999 (999999)

copy number list showing MYCN amplification



zoom-in of copy number profile showing focal amplification of MYCN



Genomics: Somatic (=tumor) variants

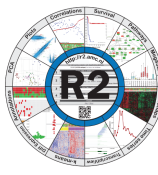
Variant Allele Frequency

Variant reads in RNA-seq

Amino acid change in protein

link	chrom	chromstart	reference	alleleseq	VAF	VarRNA	GeneSymbol	AA change	info	Logos
view	chr1	9793463	C	A	0.08		CLSTN1	p.V798L	Info	
view	chr1	150484915	C	G	0.21		ECM1	p.T266S	Info	
view	chr1	154923872	C	T	0.25		PBXIP1	p.G82S	Info	
view	chr1	156640897	C	T	0.05		NES	p.G1028R	Info	
view	chr1	169680647	T	G	0.20		SELL	p.S11R	Info	
view	chr1	235319945	G	T	0.21		RBM34	p.T139N	Info	
view	chr11	373514	C	T	0.26		B4GALNT4	p.R235W	Info	
view	chr11	47008854	C	T	0.26		C11orf49	p.T48I	Info	
view	chr11	65660758	G	C	0.24		FOSL1	p.D138E	Info	
view	chr11	70028676	G	A	0.23		ANO1	p.G825R	Info	
view	chr11	117109787	T	A	0.07		RNF214	p.D193E	Info	
view	chr12	47630113	C	T	0.05		PCED1B	p.A423V	Info	
view	chr13	36180596	T	C	0.05		NBEA	p.M2441T	Info	
view	chr16	10626883	C	T	0.54		EMP2	p.A128T	Info	
view	chr17	34305296	C	A	0.07		CCL16	p.V27F	Info	
view	chr17	40265767	G	A	0.18		KAT2A	p.R805C	Info	
view	chr17	41835999	A	T	0.09		SOST	p.I37N	Info	
view	chr17	43005466	T	A	0.11		KIF18B	p.S735C	Info	
view	chr17	76046075	G	A	0.06		TNRC6C	p.W311X	Info	
view	chr17	79409150	A	G	0.05		BAHCC1	p.D259G	Info	
view	chr19	8211062	G	GT	0.21		FBN3	p.N99fs	Info	

VAF: compare variant allele frequency with tumor % of biopsy: clonal or subclonal?

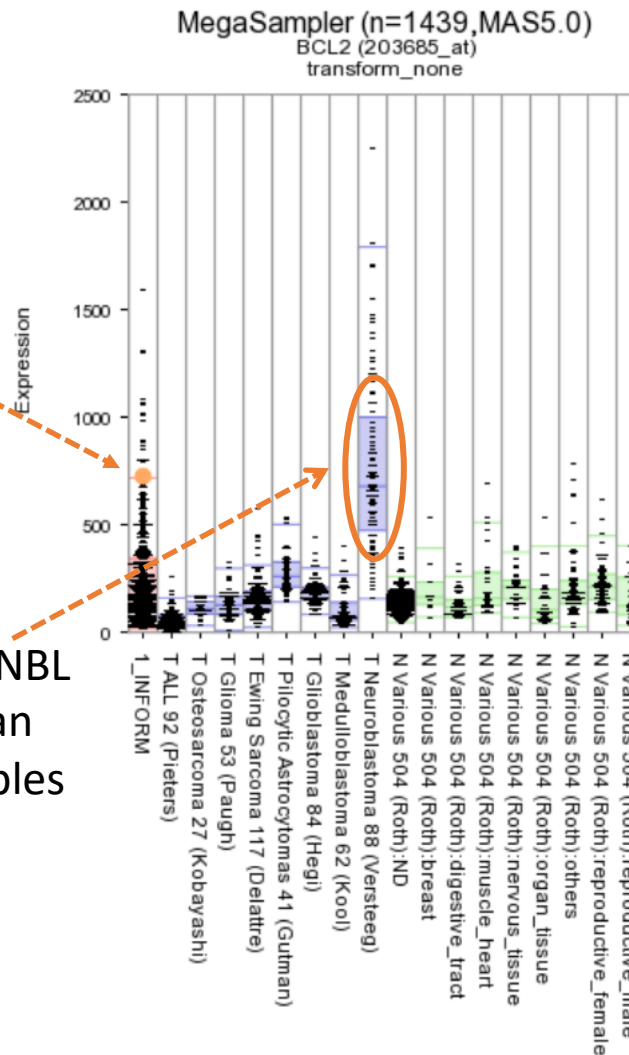


Genomics: Gene expression

BCL2 shows high gene expression in the NBL sample (orange dot)

compared with all relapse samples analyzed in the INFORM cohort

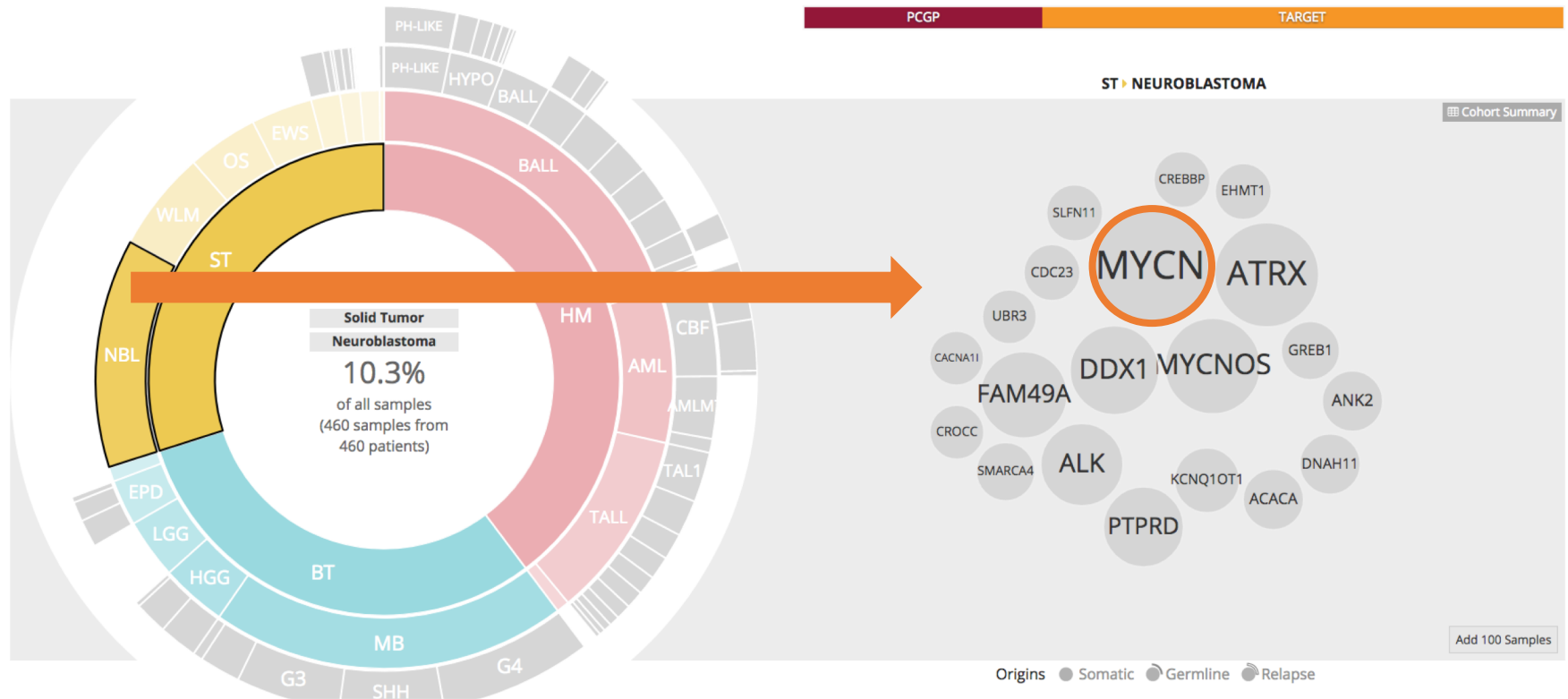
BCL2 expression in the NBL sample is around median of Neuroblastoma samples (public data)



MegaSampler view of gene expression, showing the INFORM cohort (first bar) and many public tumor (blue) and normal (green) expression cohorts

Based on Affymetrix expression microarrays

Most frequent aberrations in neuroblastoma

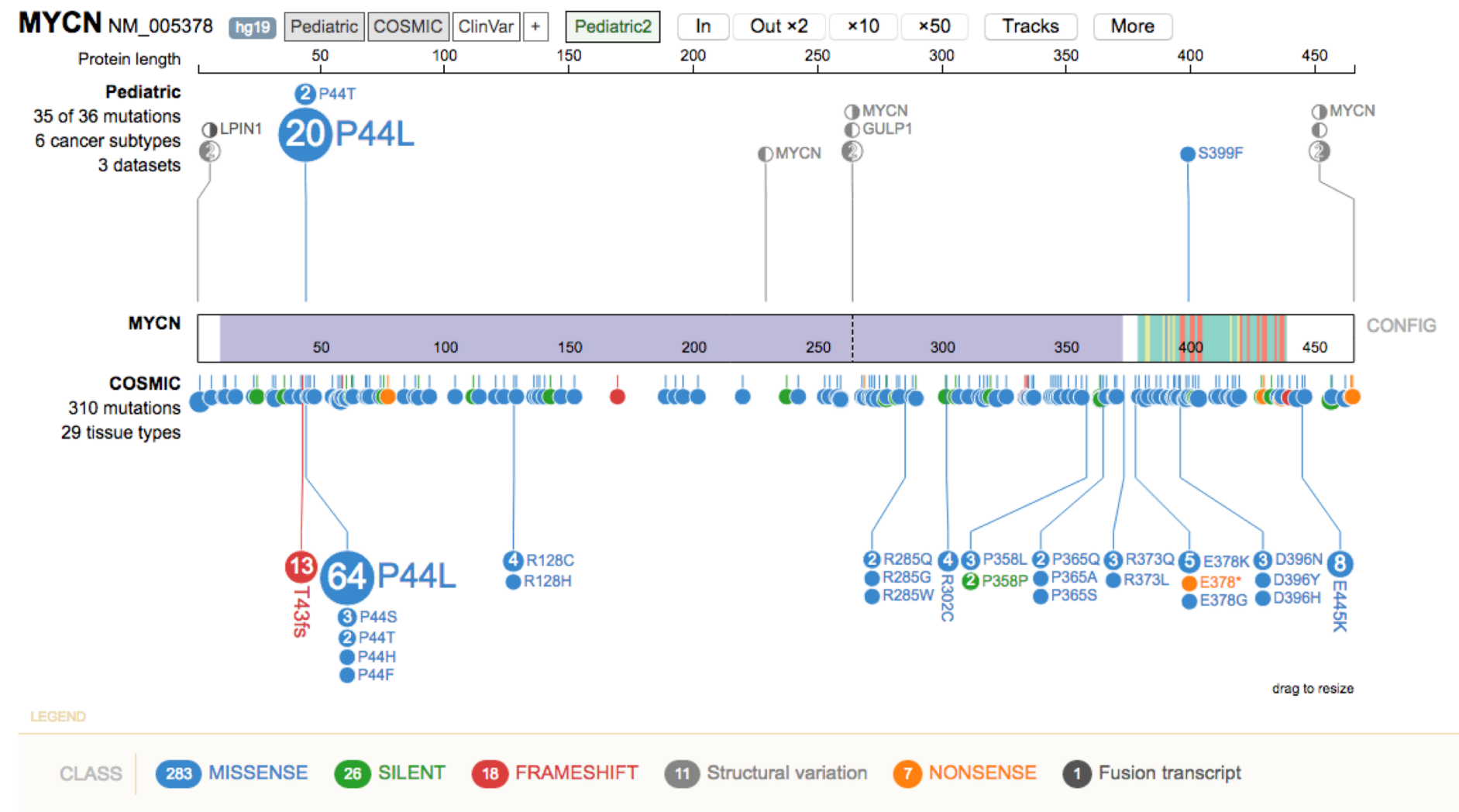


NBL: Actionable event 1

MYCN amplification (>250 copies)

- chr2:15,950,000-16,109,999
- high level focal amplification
- driving oncogene in neuroblastoma
- no direct drug target
- some evidence for increased sensitivity for several compounds (HDAC inhibitors, PI3K inhibitors, CDK7/9 inhibitors, BET inhibitors)

MYCN: oncogene often amplified/overexpressed in solid tumors and hematopoietic malignancies



MYCN signaling

Compound availability

PI3K inhibitor

- E-Smart arm F: AZD2014 + Topotecan and Temozolomide

AURKA inhibitor

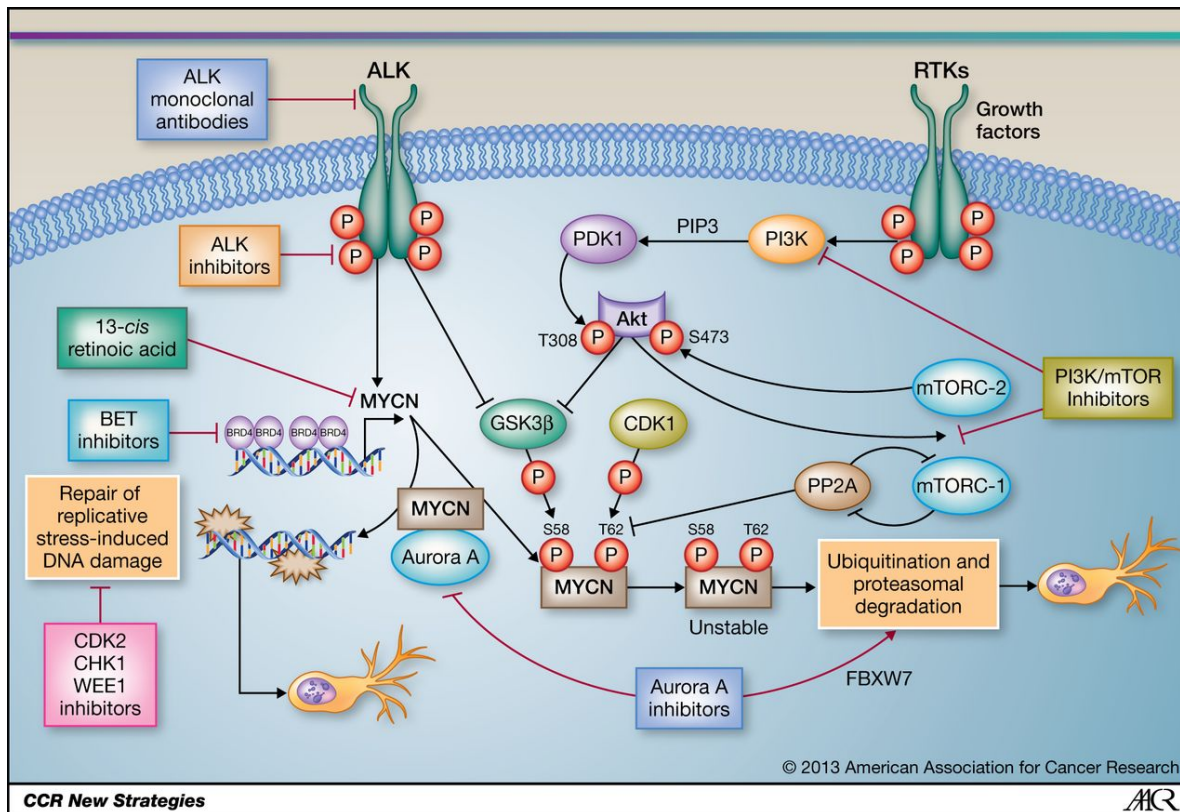
- MLN8237 combined with Irinotecan and Temozolomide (NANT trial but not recruiting)

HDAC inhibitor

- Vorinostat: Off label
- Panobinostat: Off label
- Entinostat + Nivolumab (PD1) INFORM2 trial, opening soon

BET or CDK7/9 inhibitor

- No trials in children yet



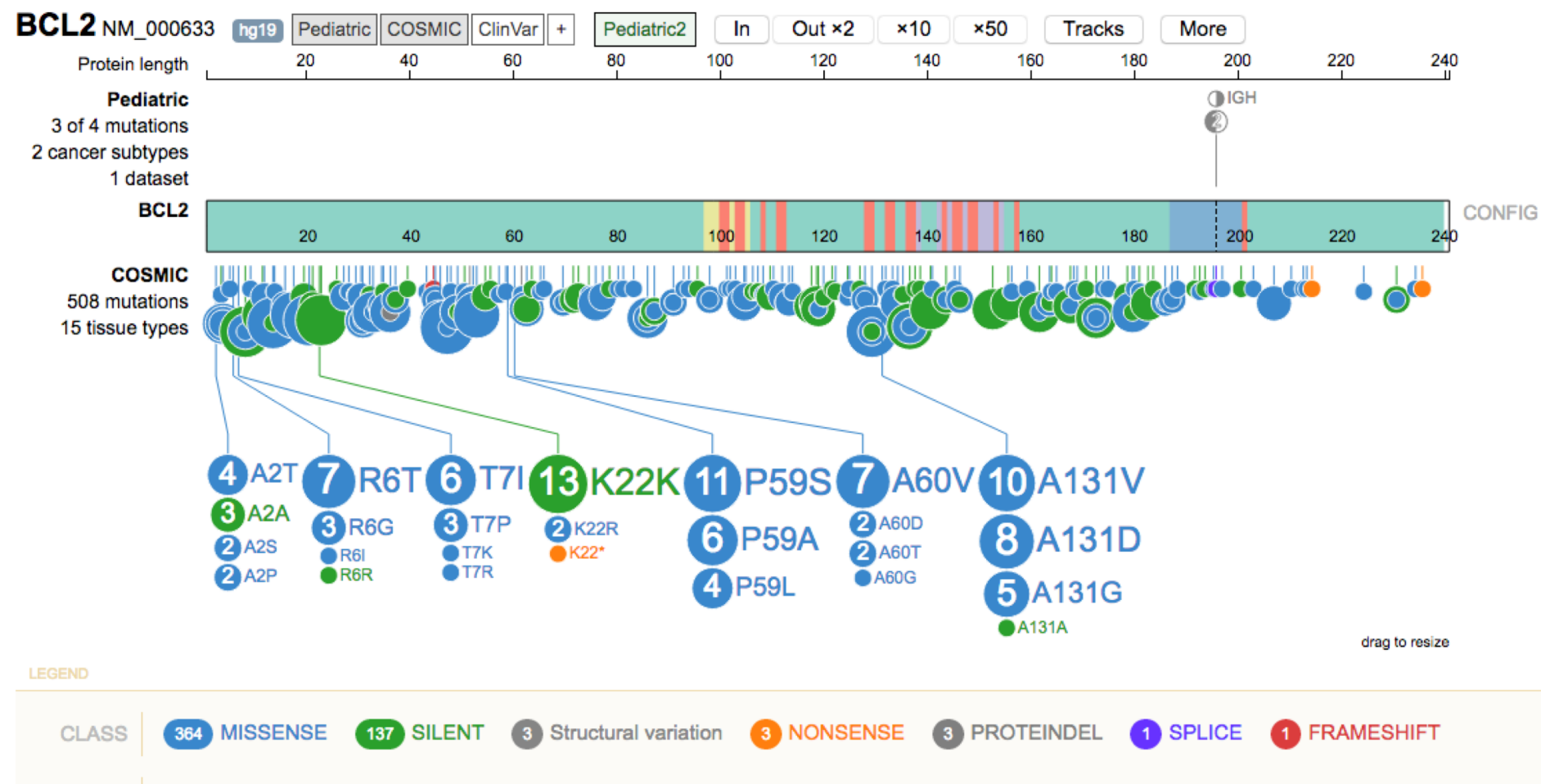
MYCN signaling in Neuroblastoma

NBL: Actionable event 2

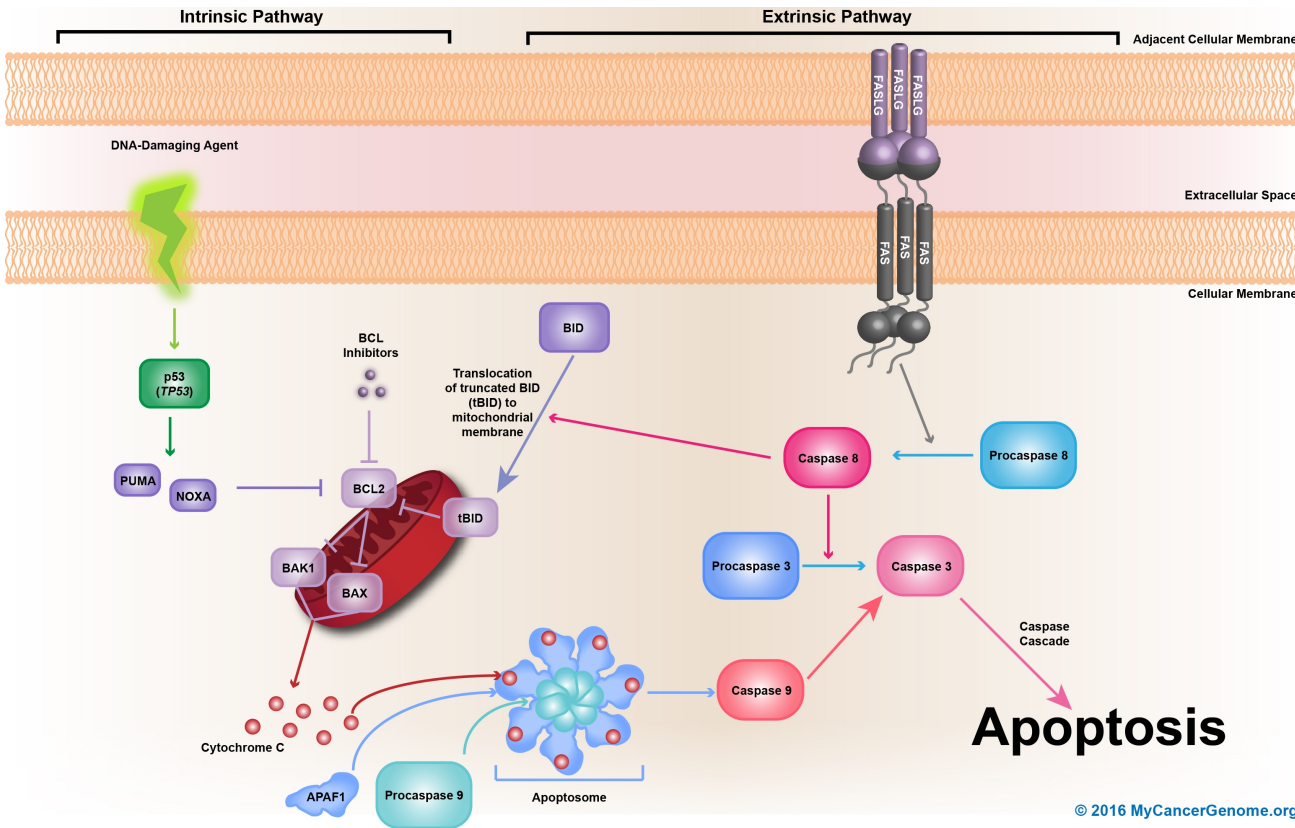
BCL2 overexpression

- Z-score 1.6 (borderline, but only 40% tumor cells); no protein data available
- no genetic alteration
- driving oncogene in neuroblastoma
- direct drug target
- evidence for sensitivity in neuroblastoma model systems

BCL2: oncogene often amplified/overexpressed in solid tumors and hematopoietic malignancies



BCL2 overexpression: blocking apoptosis



Compound availability

BCL2 inhibitor

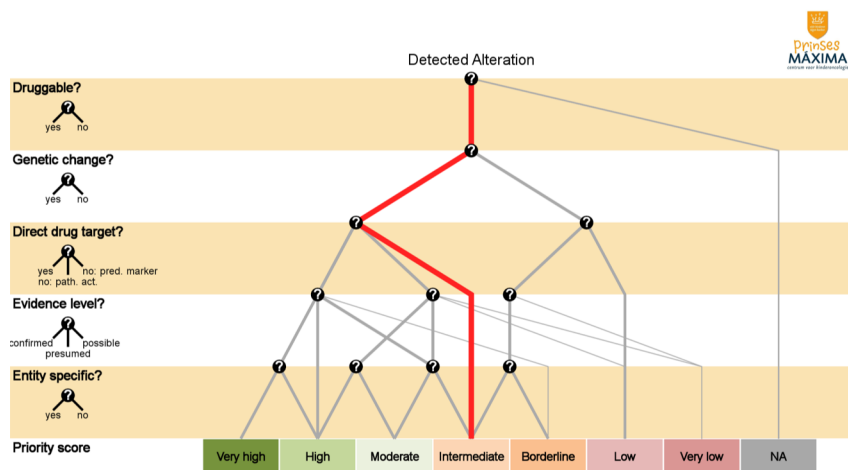
- Venetoclax: ITCC Phase 1-2 trial open, not necessary to determine BCL2 expression

BCL2 inhibitor + ALK inhibitor

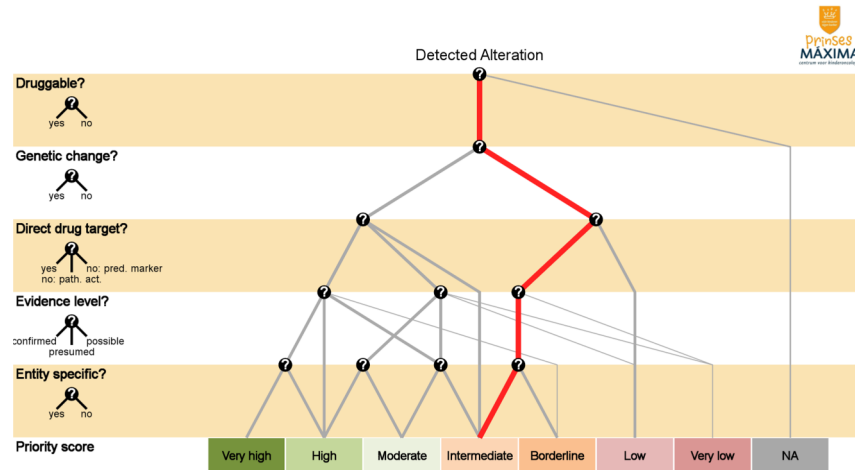
- Venetoclax combined with ALK inhibitor in vitro shows synergy. In vivo validation will be started.

Solution example 3: Neuroblastoma

Actionable event 1: MYCN amplification PI3K/AURKA/HDAC inhibitor



Actionable event 2: BCL2 overexpression BCL2 inhibitor



Alteration Type	Action of Drug	Target Type	Entity	Priority
Genetic	Sensitizing	Any	Any	4. Intermediate

Alteration Type	Action of Drug	Target Type	Entity	Priority
Expression	Direct	Confirmed	Specific	4. Intermediate

MYCN is a high risk marker but not a good actionable target. BCL2 inhibitor may work, but priority is Intermediate because the expression change is not a genetic alteration.