

ITCC EDUCATION MEETING UTRECHT

Workshop 5 – Simulated Molecular Tumor Board

Example 2

Rhabdomyosarcoma

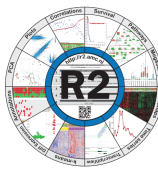
Oncologist: Clinical synopsis

- Gender: Male
- Age: 15 years
- Primary Tumor:
 - Rhabdomyosarcoma, embryonal
- History:
 - ALK negative by immunohistochemistry

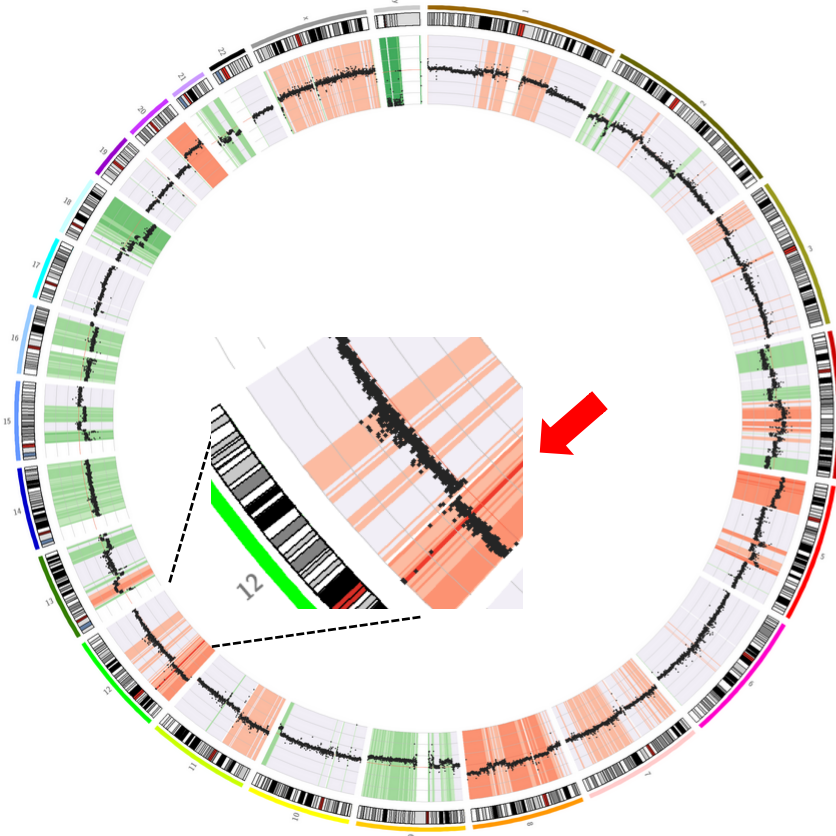
Pathologist: Tumor biopsy

- Sample: second relapse
- Type: open biopsy throat
- Quality: good, no necrotic tissue
- Tumor content: 80%
- DNA isolation: good
- RNA isolation: good

- Germline DNA: blood sample



Genomics: Copy number profile



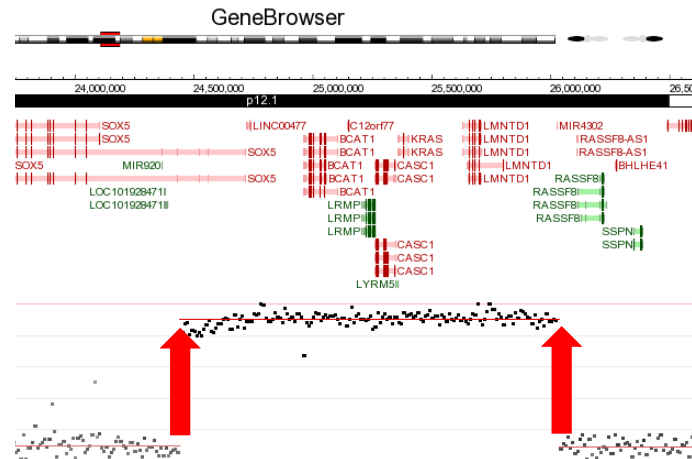
- circos plot with tracks for
- chromosome and band
 - copy number log2 ratio for tumor vs. normal (0 = diploid)

▼ CopyNumber list

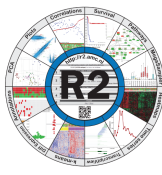
gains (red)

losses (green)

link	Gene	logfold	Info	link	Gene	logfold	Info
View	KRAS	4.4959	chr12:24440000-25439999 (999999)	View	KIR2DL3	-2.3316	chr19:55240000-55309999 (69999)
View	NRG3	1.477	chr10:84320000-84339999 (19999)	View	KIR2DL1	-2.3316	chr19:55240000-55309999 (69999)
View	ERBB4	0.9903	chr2:213130000-213169999 (39999)	View	KIR3DL2	-2.3316	chr19:55320000-55369999 (49999)
View	TERT	0.9769	chr5:1010000-2009999 (999999)	View	BCL2	-1.273	chr18:60490000-61489999 (999999)
View	PTK2	0.7896	chr8:141990000-142759999 (769999)	View	PHLPP1	-1.273	chr18:59490000-60489999 (999999)
View	SRC	0.7825	chr20:35940000-36419999 (479999)	View	CD38	-0.7077	chr4:15800000-16799999 (999999)
View	MYC	0.7695	chr8:128210000-129209999 (999999)	View	SLC34A2	-0.6741	chr4:25340000-26339999 (999999)
View	CD40	0.7256	chr20:44220000-45219999 (999999)	View	VEGFC	-0.6184	chr4:177280000-178279999 (999999)
View	MMP9	0.7256	chr20:44220000-45219999 (999999)	View	ERG	-0.5978	chr21:39650000-40649999 (999999)



zoom-in of
copy number
profile
showing **focal
amplification
of KRAS**



Genomics: Gene expression

(sample_id=553)
Using Genelist: def: grp.ither.ither_genelist_v2018

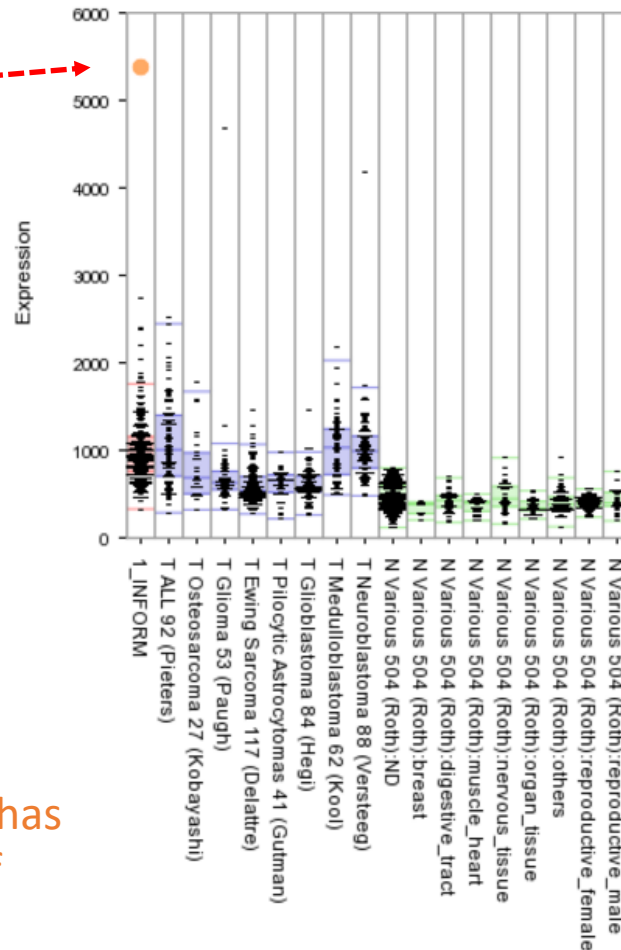
Plot Top10 Genes

1GeneView	Gene	zscore	Detailed
204009 s at	KRAS	4.642	Detailed
208694 at	PRKDC	2.484	Detailed
210229 s at	CSF2	2.298	Detailed
204579 at	FGFR4	2.258	Detailed
210416 s at	CHEK2	2.143	Detailed
201209 at	HDAC1	1.802	Detailed
209464 at	AURKB	1.732	Detailed
207145 at	MSTN	1.730	Detailed
202288 at	MTOR	1.669	Detailed
213523 at	CCNE1	1.667	Detailed
212348 s at	KDM1A	1.620	Detailed
32137 at	JAG2	1.591	Detailed
210038 at	PRKQC	1.539	Detailed

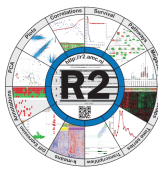
Gene expression list ranking
Affymetrix-based expression
values relative to all relapse
samples analyzed in INFORM

This RMS sample (orange dot)
has the highest KRAS expression of
the INFORM study samples

MegaSampler (n=1297, MAS5.0)
KRAS (204009_s_at)
transform_none



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



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	6184676	G	A	0.27	0.1	CHD5	p.A1480V	Info	
view	chr1	9324455	G	C	0.04	0.1	H6PD	p.G635A	Info	
view	chr1	19468154	C	A	0.61	22	UBR4	p.D2794Y	Info	
view	chr1	24082462	T	C	0.27	55	TCEB3	p.L667P	Info	
view	chr1	114308738	A	G	0.20	16	RSBN1	p.S758P	Info	
view	chr1	119964914	A	G	0.25	0.1	HSD3B2	p.Y264C	Info	
view	chr1	179078242	C	T	0.12	0.1	ABL2	p.G699E	Info	A
view	chr1	197073482	C	T	0.49	18	ASPM	p.R1633H	Info	C
view	chr1	231155818	G	C	0.54	29	FAM89A	p.Y115X	Info	C
view	chr1	240939505	G	T	0.53	112	RGS7	p.S456Y	Info	C
view	chr10	29581493	G	T	0.09	0.1	LYZL1	p.Q108H	Info	
view	chr10	56138561	C	T	0.28	0.1	PCDH15	p.G100R	Info	C
view	chr11	6292263	T	C	0.19	0.1	CCKBR	p.C279R	Info	
view	chr11	61099034	T	A	0.33	65	DDB1	p.M64L	Info	
view	chr11	74204451	G	T	0.27	2	LIPT2	p.C99X	Info	
view	chr11	82703417	C	G	0.27	0.1	RAB30	p.R3T	Info	
view	chr11	92087759	AG	A	0.25	0.1	FAT3	p.S828fs	Info	
view	chr16	11556157	C	A	0.41	0.1	CTD-3088G3.8	p.G2V	Info	
view	chr17	4996218	G	A	0.10	2	ZFP3	p.E474K	Info	
view	chr17	7577558	G	A	0.70	215	TP53	p.S241F	Info	C
view	chr17	8092791	G	C	0.89	21	C17orf59	p.L223V	Info	
view	chr17	12661516	A	G	0.27	1	MYOCD	p.D725G	Info	
view	chr17	17701077	G	A	0.29	21	RAI1	p.A1606T	Info	
view	chr17	28407768	A	G	0.33	0.1	EFCAB5(ENST00000320856.5)	p.E70X	Info	
view	chr17	40812211	G	T	0.46	5	TUBG2	p.E70X	Info	
view	chr17	76212769	G	C	0.29	122	AC087645.1,BIRC5	p.G83R	Info	
view	chr18	29419296	T	A	0.14	20	TRAPPC8	p.N1321Y	Info	
view	chr18	47801590	C	T	0.33	51	MBD1	p.G273S	Info	
view	chr19	2115310	C	G	0.27	56	AP3D1	p.K752N	Info	
view	chr19	4432077	GACAGA	G	0.31	13	CHAF1A	p.693_694del	Info	
view	chr19	8191658	C	T	0.32	0.1	FBN3	p.G785D	Info	
view	chr19	9024868	C	A	0.60	0.1	MUC16	p.E12331D	Info	A
view	chr19	10404755	G	A	0.28	3	ICAM5	p.W584X	Info	

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

VarRNA: indication of expression level of variant allele

OncoKB database has information on **TP53** mutation:

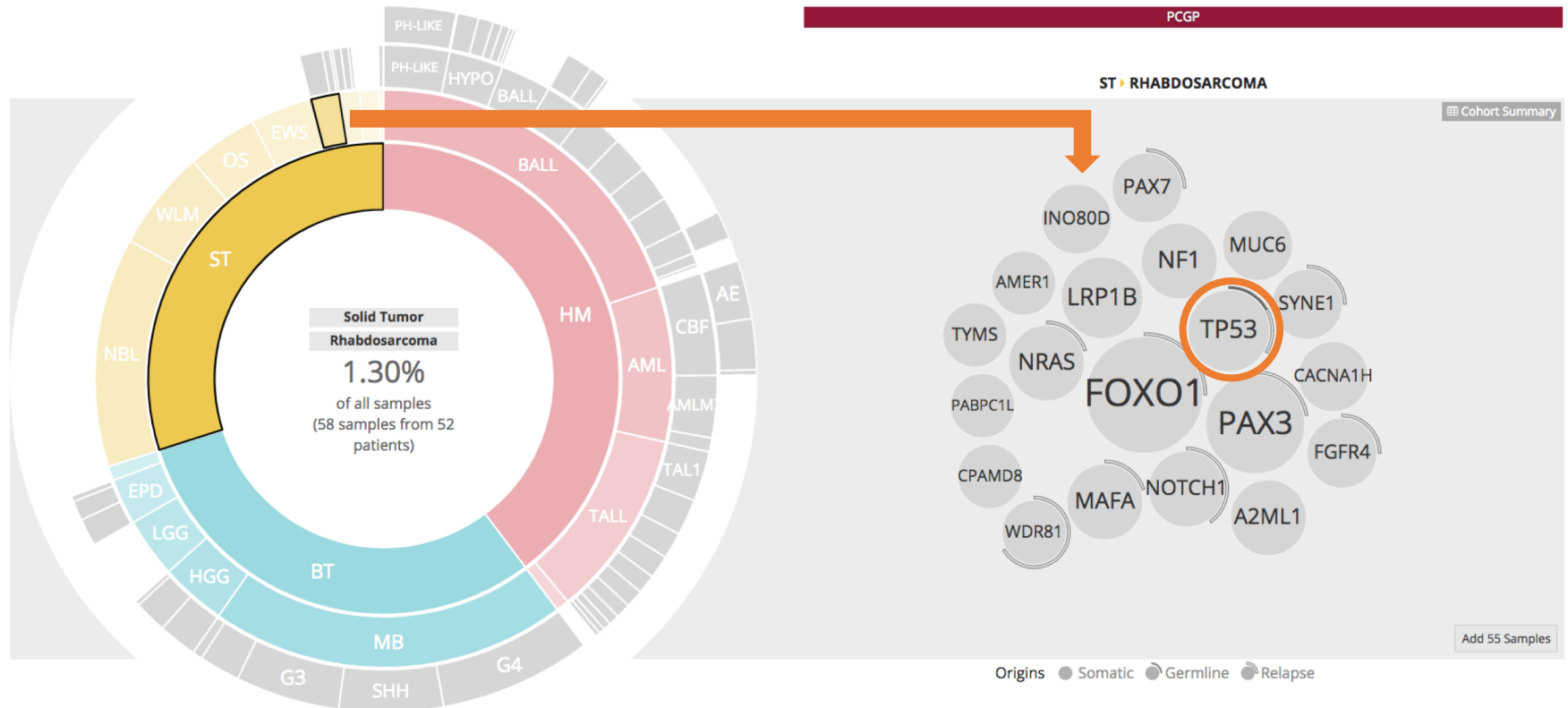
▼ OncoKB (1 hits)			
All Variants			
Gene Alteration	Oncogenicity	Mutation Effect	
TP53 S241F	Likely Oncogenic	Likely Loss-of-function	

C: Cosmic Mutation

A: Actionable Gene (according to [grp.ither.ither_genelist_v2018](#))

VAF 0.10: VAF <=0.1

Most frequent aberrations in rhabdosarcoma

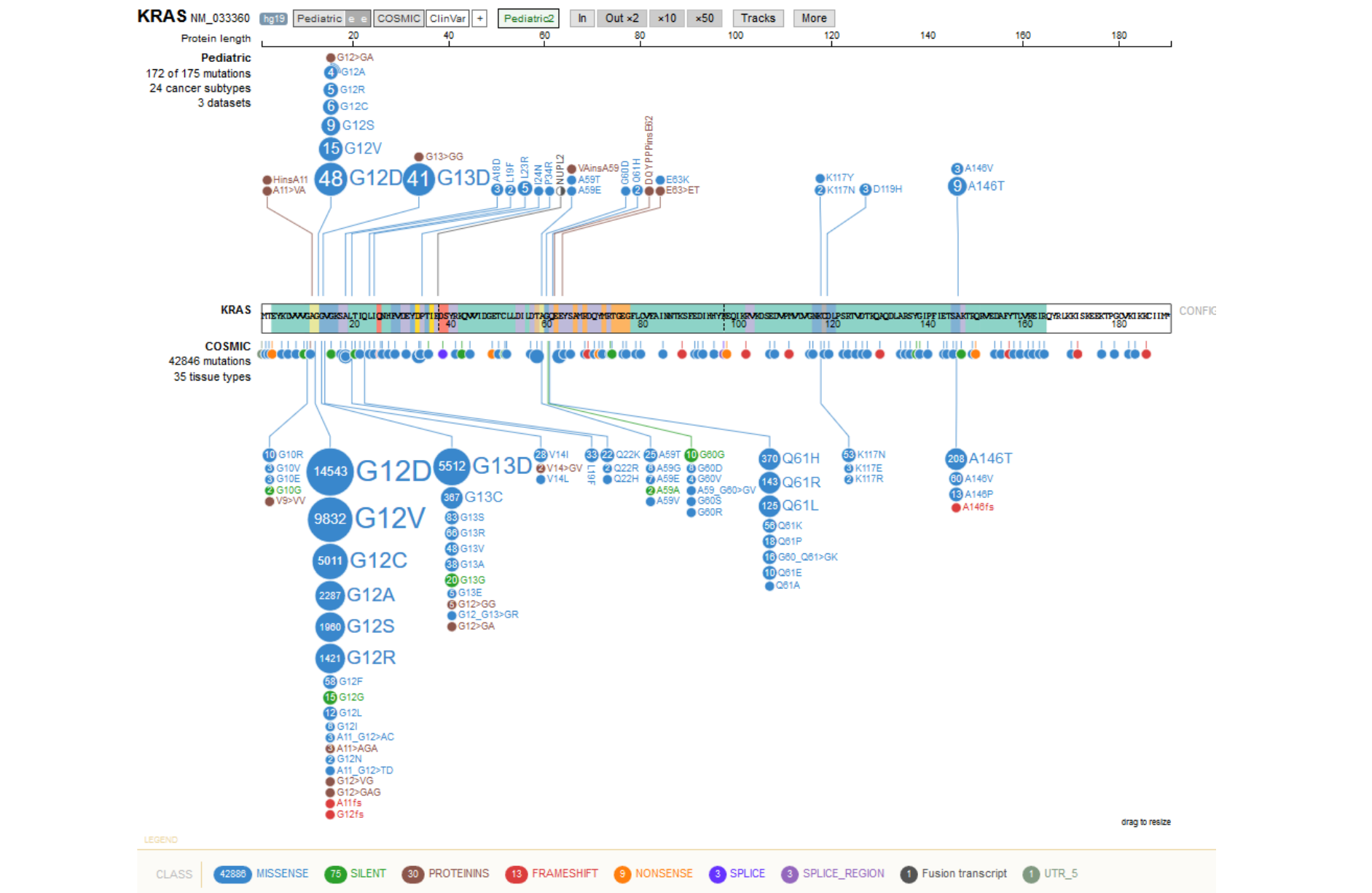


RMS: Actionable event 1

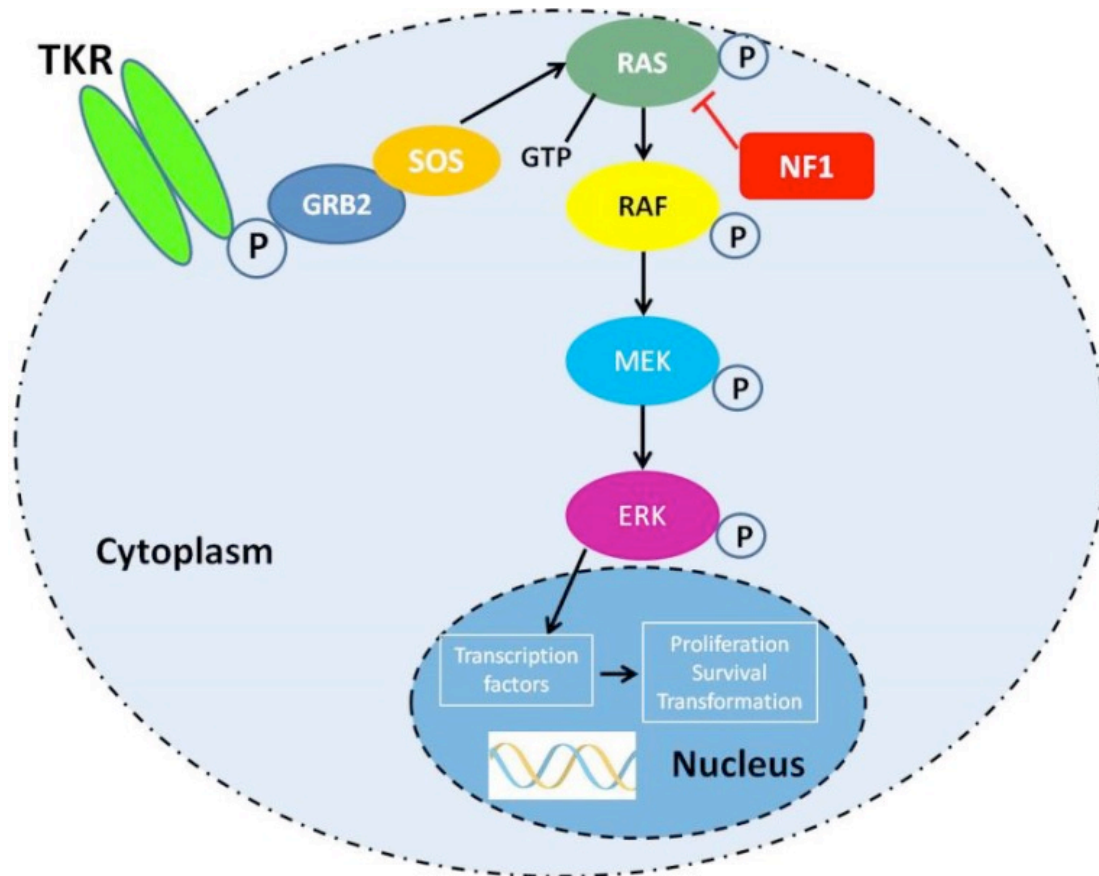
KRAS amplification (>32 copies) and overexpression (zscore 4.8)

- chr12:25,204,789-25,250,936
- focal amplification log2 ratio 4.5
- mutations well studied, but effect of amplification less known; KRAS is also very highly expressed
- activating KRAS mutations are described in RMS
- RAS pathway can be inhibited downstream (MEK)

KRAS: oncogene often mutated in all types of malignancies



RAS-RAF-MEK-ERK signaling



Compound availability

MEK1/2 inhibitors

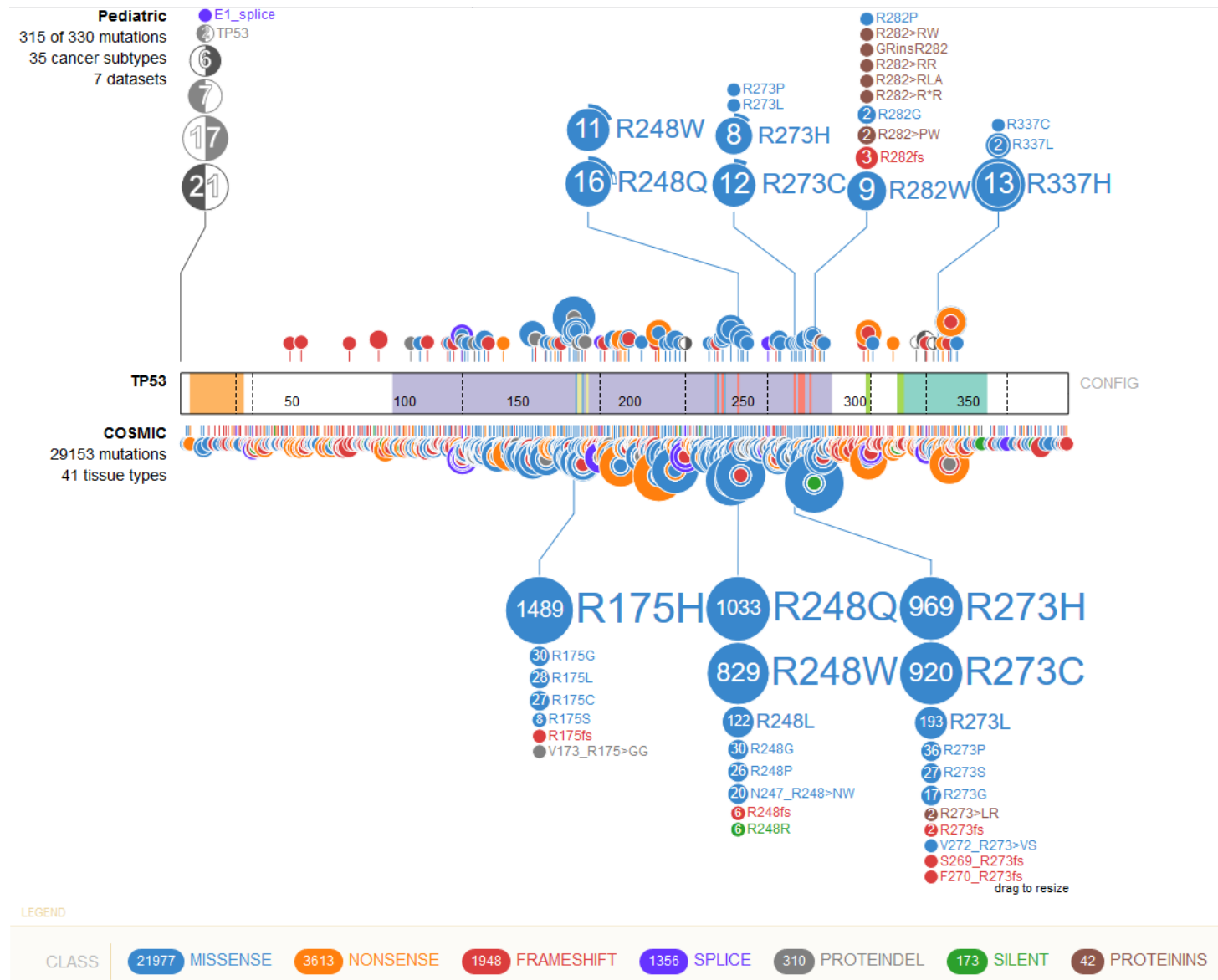
- Cobimetinib: Phase 1/2 ITCC055, open in FR, DE and UK
- Cobimetinib: Off label
- Trametinib: Off label, dose finding in children is ongoing in Neuroblastoma trial in Philadelphia
- Selumetinib: Off label
- Binimetinib: Off label, Ph 1/2 ongoing in US

RMS: Actionable event 2

TP53 mutation p.S241Y

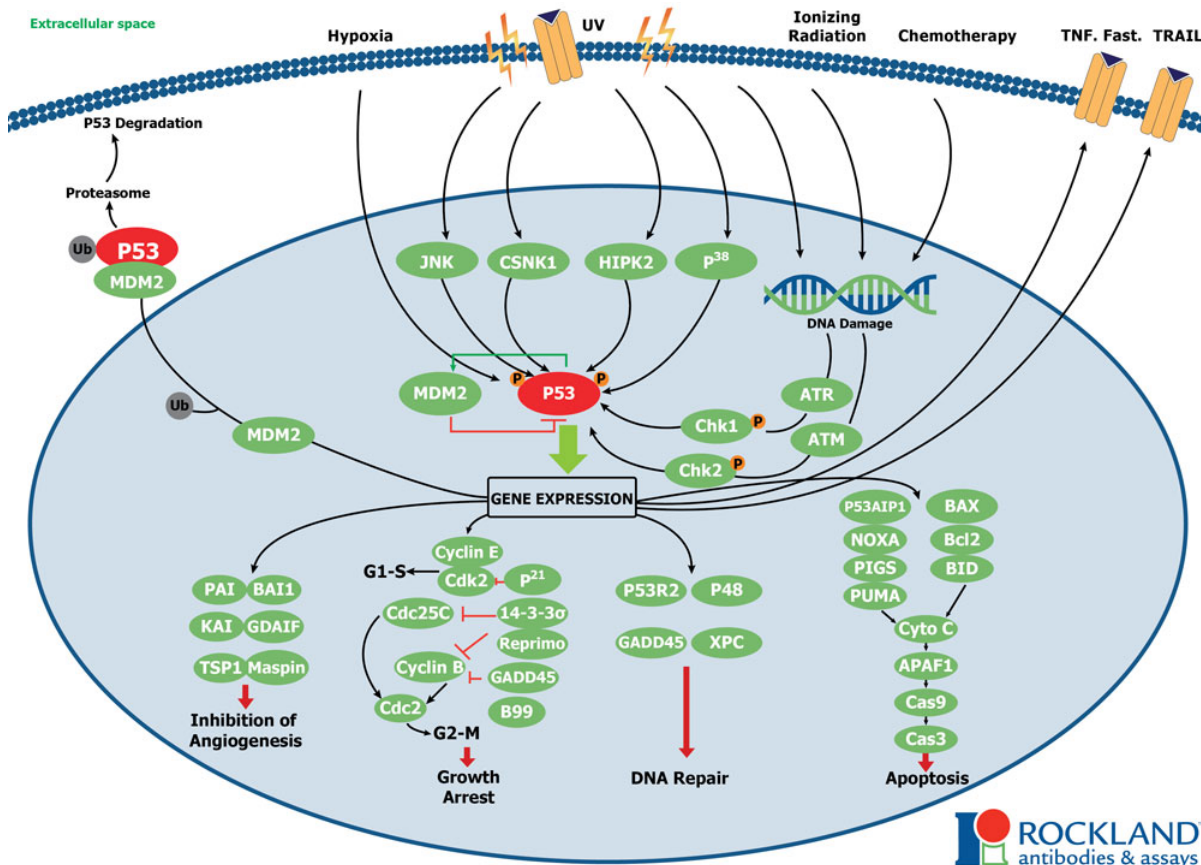
- chr17:7,577,558
- Protein: missense Serine241Phenylalanine
- Likely loss-of-function mutation
 - Variant allele frequency DNA 0.70, RNA 0.92 → probably no wildtype allele
- Well established tumor suppressor event in many cancer types, including RMS
- Of biological interest, but currently not targetable

TP53: tumor suppressor gene often deleted or mutated in all types of malignancies



Source: Pediatric Cancer Database, St. Jude: pecan.stjude.org

TP53 signaling

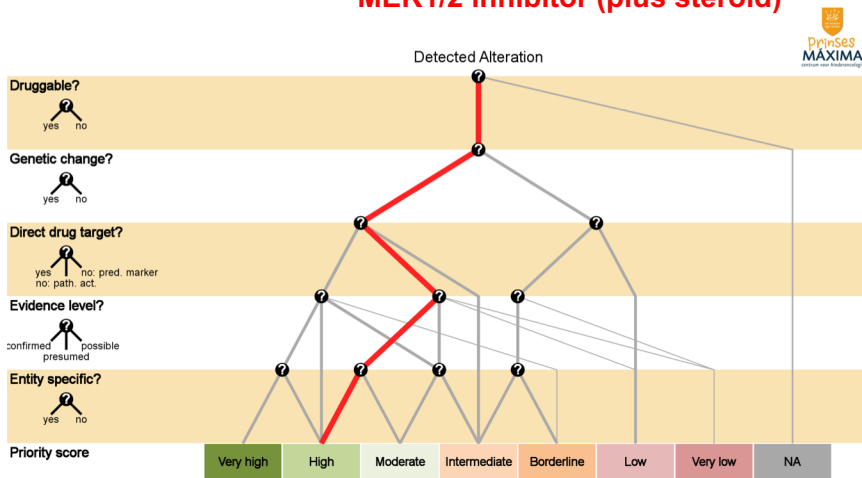


Compound availability

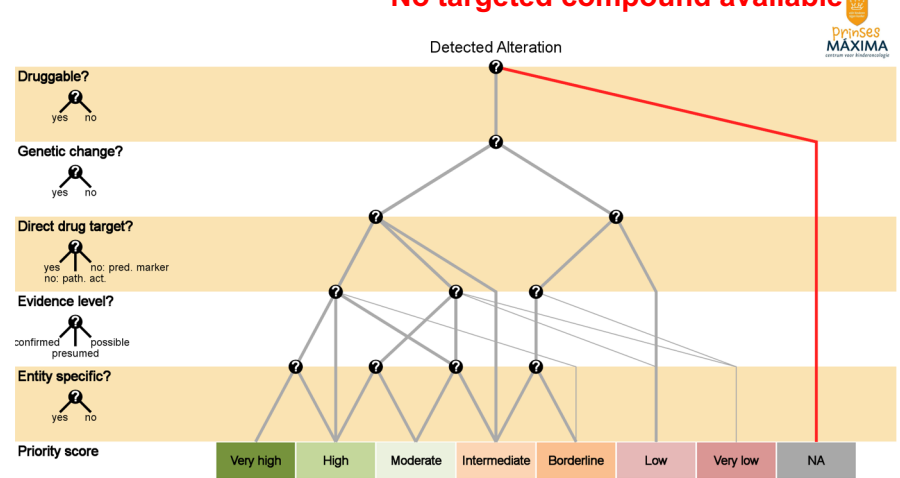
No MDM2 inhibitor
No targeted compound
available

Solution example 2: RMS

Actionable event 1: **KRAS amplification** **MEK1/2 inhibitor (plus steroid)**



Actionable event 2: **TP53 mutation** **No targeted compound available**



Alteration Type	Action of Drug	Target Type	Entity	Priority
Genetic	Pathway	Confirmed pathway activation	Specific	2. High

Alteration Type	Action of Drug	Target Type	Entity	Priority
Genetic	Any	Biological interest	Any	8. NA

KRAS amplification is thought to have a similar oncogenic effect as the more frequently occurring NRAS mutation in rhabdomyosarcoma.

The TP53 mutation was somatic; reminder that sequencing can uncover germline mutations associated with cancer predisposition syndromes (e.g. Li-Fraumeni).