

ITCC EDUCATION MEETING UTRECHT

Workshop 5 – Simulated Molecular Tumor Board

Example 1

Acute lymphoblastic leukemia

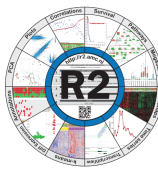
Oncologist: Clinical synopsis

- Gender: Male
- Age: 9 years
- Primary Tumor:
 - acute lymphoblastic leukemia, T-ALL
- History:
 - medium risk treatment, relapse during treatment
 - resistant disease on relapse protocol and NECTAR

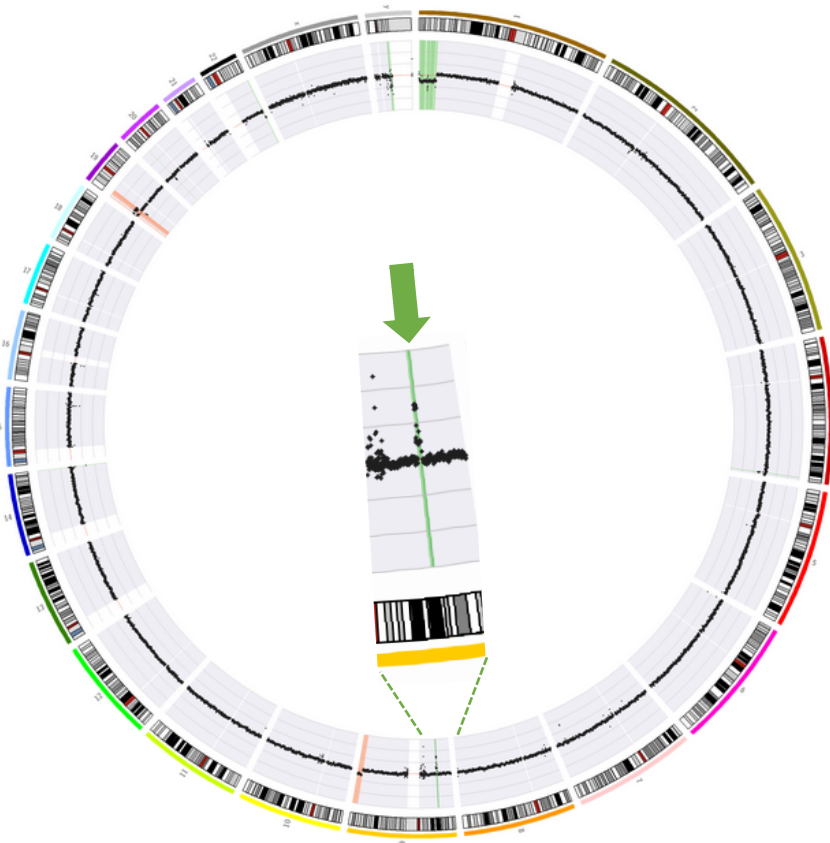
Pathologist: Tumor biopsy

- Sample: relapse
- Type: bone marrow aspiration
- Quality: good
- Tumor content: 88%
- DNA isolation: good
- RNA isolation: good

- Germline DNA: remission sample



Genomics: Copy number profile

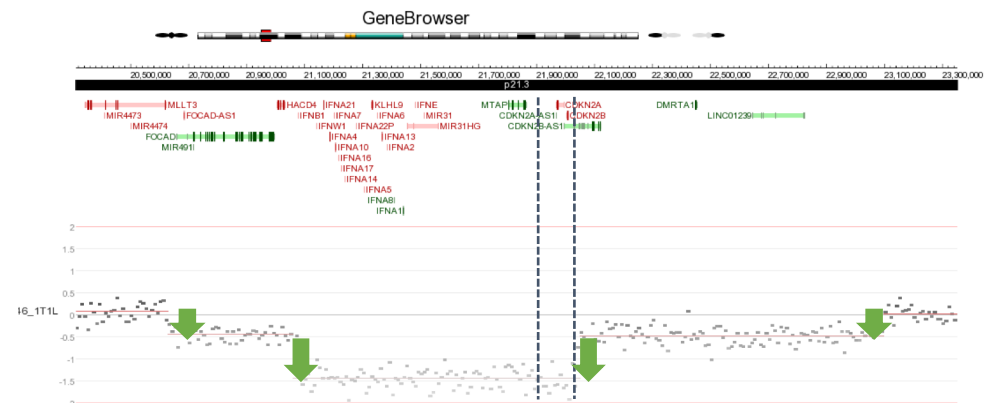


gains (red)

losses (green)

link	Gene	logfold	Info	link	Gene	logfold	Info
View	CD70	0.4781	chr19:6300000-7029999 (729999)	View	CDKN2B	-1.4363	chr9:21060000-22049999 (989999)
View	DNMT1	0.4767	chr19:9730000-10519999 (789999)	View	CDKN2A	-1.4363	chr9:21060000-22049999 (989999)
View	TYK2	0.4767	chr19:9730000-10519999 (789999)	View	HDAC10	-0.5304	chr22:50410000-51249999 (839999)
View	MUC16	0.4767	chr19:8730000-9729999 (100000)	View	PIM3	-0.5304	chr22:50200000-50369999 (160000)

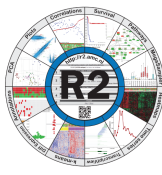
copy number list showing CDKN2A/B loss



zoom-in of copy number profile showing **focal bi-allelic deletion of CDKN2A/B** (between the vertical lines)

circos plot with tracks for

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



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	1258559	C	T	0.50	0	CPSF3L	p.V46M	Info	
view	chr1	23720426	C	G	0.44	4	TCEA3	p.R255P	Info	
view	chr1	27874287	G	A	0.30	5	AHDC1	p.R1447W	Info	
view	chr1	43869373	C	T	0.05	3	SZT2	p.R86W	Info	
view	chr1	65304232	G	C	0.33	20	JAK1	p.S961W	Info	A
view	chr1	175360440	G	A	0.54	0	TNR	p.S497L	Info	C
view	chr11	67052452	C	T	0.08	49	ADRBK1	p.P597L	Info	C
view	chr16	1968148	G	A	0.11	0	HS3ST6	p.A28V	Info	
view	chr17	79682594	G	A	0.34	13	SLC25A10	p.E101K	Info	
view	chr19	10935853	G	A	0.46	89	DNM2	p.R672H	Info	C
view	chr19	38964169	AC	A	0.24	0	RYR1	p.T1307fs	Info	
view	chr4	80329256	G	A	0.34	0	GK2	p.A33V	Info	C
view	chr5	35874569	A	ATG	0.16	0	IL7R	p.L243fs	Info	A
view	chr5	79375860	G	A	0.37	0	THBS4	p.R882H	Info	
view	chr5	140167546	G	A	0.34	0	PCDHA1	p.E558K	Info	C
view	chr6	137245339	G	A	0.07	0	SLC35D3	p.V253M	Info	
view	chr7	140082250	G	A	0.29	12	SLC37A3	p.L26F	Info	
view	chr7	142028604	TG	T	0.31	0	TRBV6-1	p.E114fs	Info	
view	chr8	56675159	A	G	0.27	1	TMEM68	p.I120T	Info	
view	chr8	81899675	C	T	0.11	8	PAG1	p.R68H	Info	
view	chr8	128750672	G	GGTCGCC	0.17	8	MYC	p.L71delinsRRL	Info	A
view	chr9	32784796	G	A	0.30	0	TMEM215	p.V206I	Info	
view	chr9	139390863	C	CCCCGA	0.35	40	NOTCH1	p.D2442fs	Info	A
view	chr9	139399355	A	T	0.47	65	NOTCH1	p.L1596H	Info	C A

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

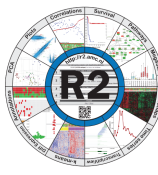
VarRNA: frame shift (fs) mutations are not (yet) annotated in the RNA, number of reads in RNA-seq is zero

Subclonal **IL7R** mutation, compound heterozygous NOTCH1 mutations, JAK1 mutation, MYC mutation, etc.

C: Cosmic Mutation

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

VAF 0.10: VAF <=0.1

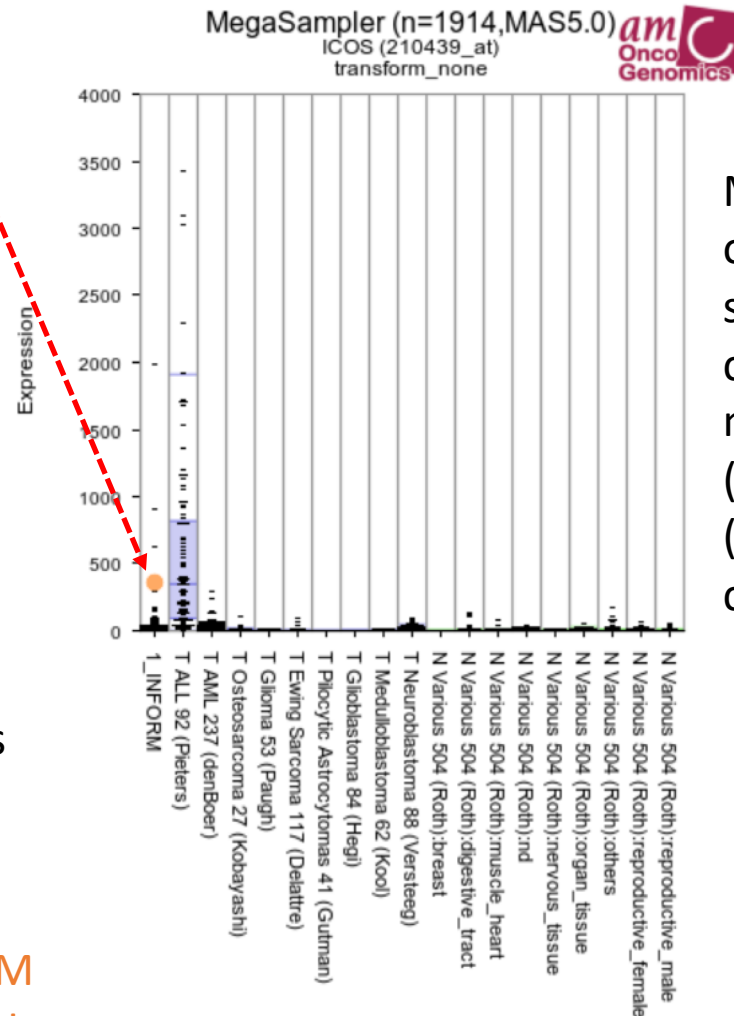


Genomics: Gene expression

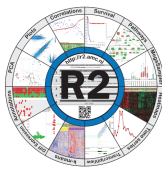
1GeneView	Gene	zscore	Detailed
210439 at	ICOS	4.061	Detailed
218764 at	PRKCH	3.871	Detailed
208438 s at	FGR	3.318	Detailed
204891 s at	LCK	2.617	Detailed
211339 s at	ITK	2.564	Detailed
204661 at	CD52	2.553	Detailed
209124 at	MYD88	2.483	Detailed
208018 s at	HCK	2.451	Detailed
201209 at	HDAC1	2.422	Detailed
210038 at	PRKCQ	2.327	Detailed
206369 s at	PIK3CG	2.276	Detailed
205692 s at	CD38	2.265	Detailed

Gene expression list ranking
Affymetrix-based expression values

This T-ALL sample (orange dot) has high ICOS expression relative to all relapse samples analyzed in INFORM but normal relative to an ALL cohort



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



Genomics: Gene fusions

ARRIBA (INFORM / iTHER)

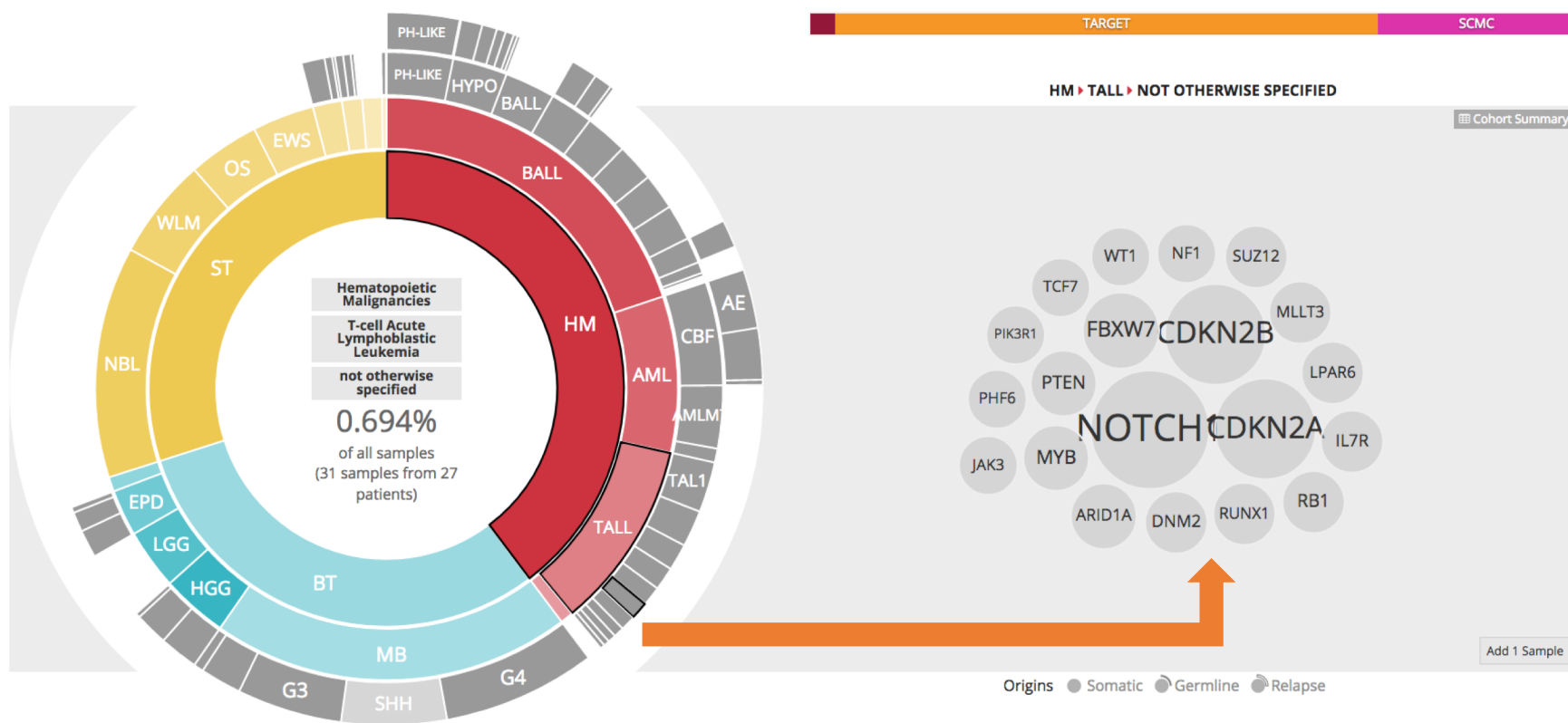
gene1	gene2	breakpoint1	breakpoint2	confidence
ADRBK2	IGLL3P	22:25961121	22:25696682	high
TLX1	TLX1	10:102893382	10:102891092	high

Two high confidence gene fusions detected by ARRIBA

Breakpoints are close, these are probably read-through events or intragenic deletion

No oncogenic fusion gene detected

Most frequent aberrations in T-cell acute lymphoblastic leukemia (not otherwise specified)



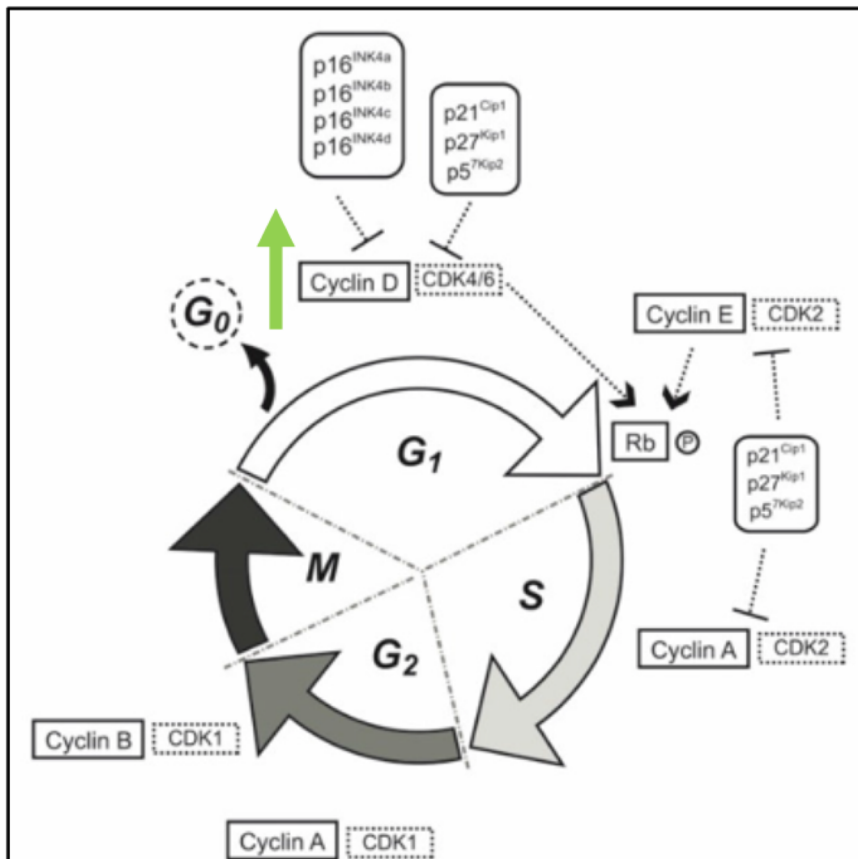
T-ALL: Actionable event 1

CDKN2A/B homozygous deletion

- chr9: 21,060,000-22,049,999
- genetic change: copy number log2 ratio -1.4
- biallelic loss leading to complete inactivation
- tumor suppressor gene frequently deleted in T-ALL
- direct repressor of CDK4/6, deletion leads to activation of the cell cycle
- preclinical data show sensitivity to CDK4/6 inhibitor in cell lines

Cell cycle: cyclin D-CDK4/6 activation

Cyclin Dependent Kinase Inhibitor 2A (CDKN2A) encodes p16INK4A, which represses Cyclin Dependent Kinases CDK4/6



Compound availability

CDK4/6 inhibitors

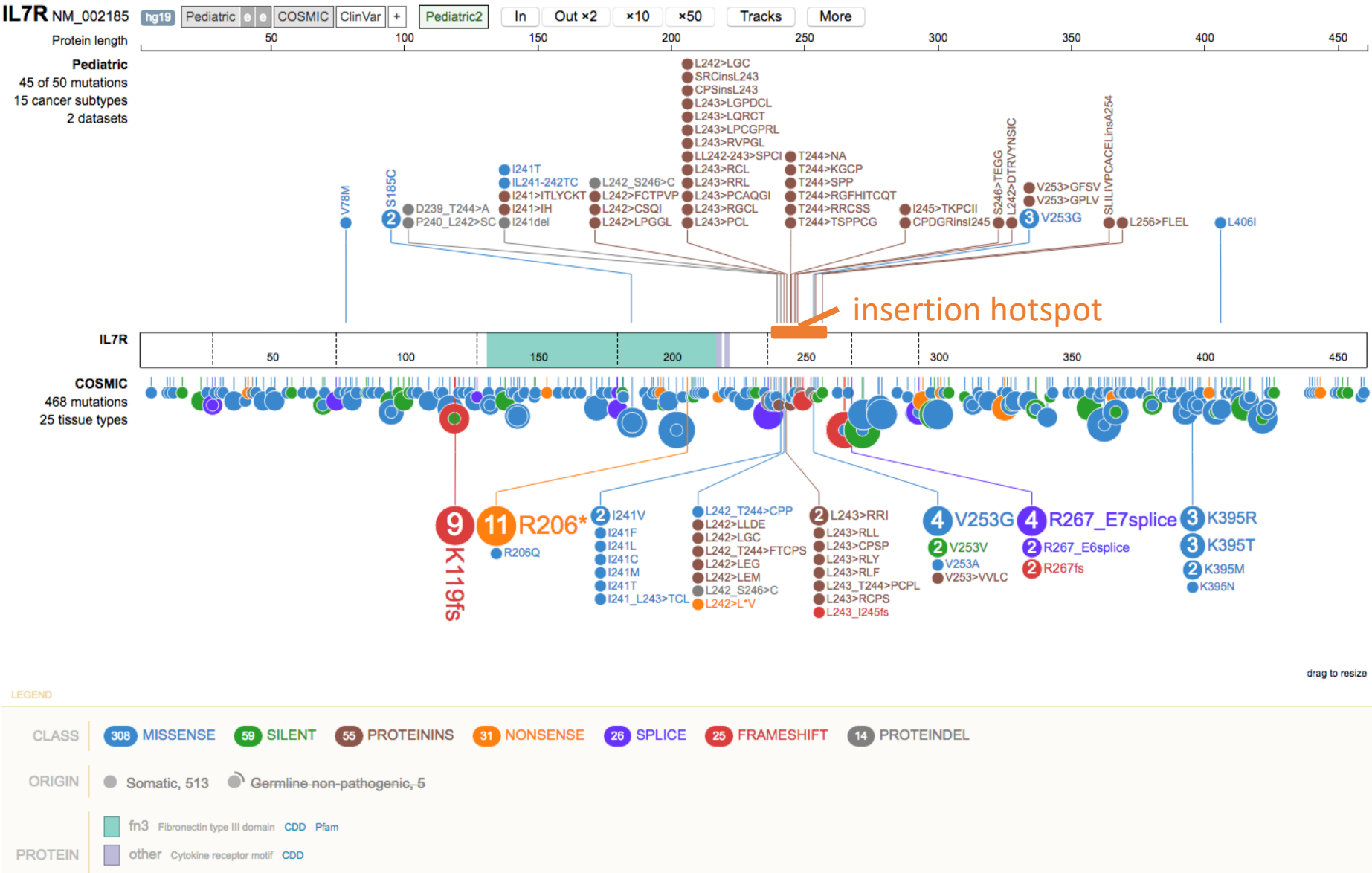
- Ribociclib: **Ph1/2 in E-Smart (NCT02813135)** selection biomarkers include: CDKN2A and/or CDKN2B deletion, CDK4/6 amplification
 - Arm A: ribociclib + toptecan and temozolomide (TOTEM)
 - arm B: ribociclib + everolimus (MTOR inhibitor)
- Ribociclib: Off label
- Palbociclib: Off label
- Abecaciclib: Not yet available

T-ALL: Actionable event 2

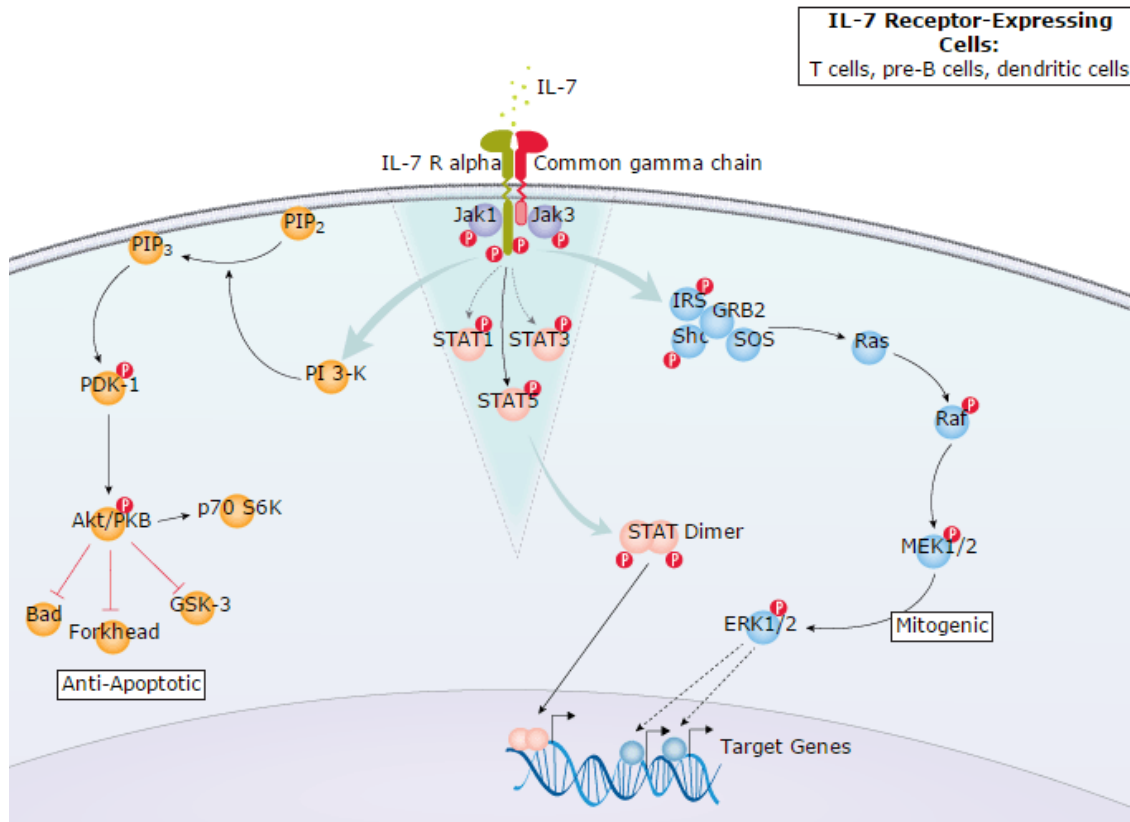
IL7 receptor mutation

- chr5:35,874,569
- protein insertion: Leu242_Leu243insCysProSer
- in-frame cysteine introduced: presumed activating
- hotspot in T-ALL for INDELS (Zernatti et al. Nature Genetics 2011)
- may be sensitive to MEK, AKT, mTOR, or PI3K inhibitors combined with steroid (prednisone or dexamethasone)
- preclinical data on patients' cells exposed *ex vivo*

IL7R: oncogene often mutated in hematopoietic malignancies



IL7 receptor signaling



Compound availability

MEK1/2 inhibitors

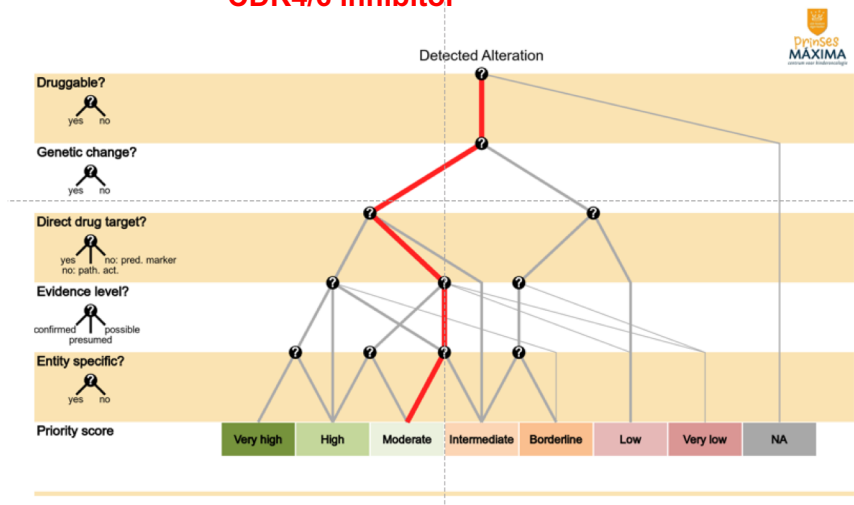
- Cobimetinib: Phase 1/2
ITCC055, open in FR, DE and UK
- Cobimetinib: Off label
- Trametinib: Off label, dose finding in children is ongoing in Neuroblastoma trial in Philadelphia
- Selumetinib: Off label
- Binimetinib: Off label, Ph 1/2 ongoing in US

MEK1/2 inhibitors + steroid

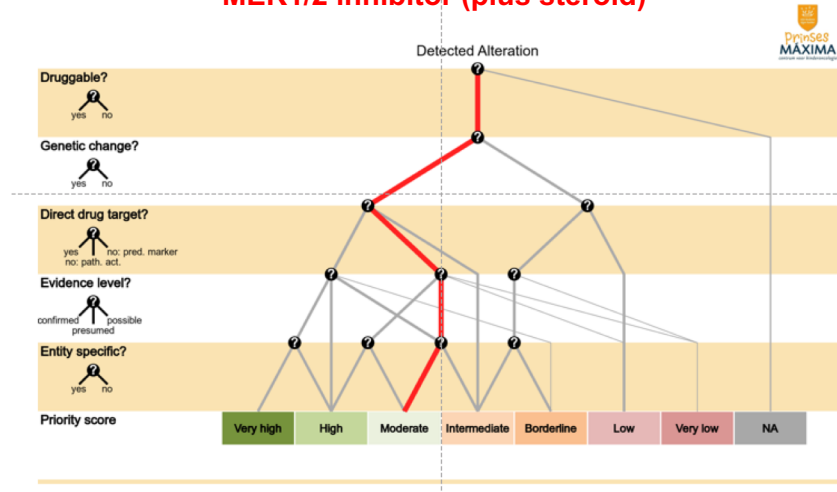
- Plan to open soon: Seludex study, combining Selumetinib with Dexamethasone; selection biomarker: RAS-pathway mutation

Solution example 1: T-cell ALL

Actionable event 1: CDKN2A/B homozygous deletion CDK4/6 inhibitor



Actionable event 2: IL7R mutation MEK1/2 inhibitor (plus steroid)



Alteration Type	Action of Drug	Target Type	Entity	Priority
Genetic	Pathway	Presumed pathway activation	Specific	3. Moderate

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The actionable events are probably not the primary oncogenic driver; treatment with single compound likely to result in resistance. Patient is being treated with standard chemotherapy to bridge to stem cell transplantation.