# Clinical Application of Cancer Genomics

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**ITCC INTRODUCTORY COURSE**

**IN PAEDIATRIC DRUG DEVELOPMENT 2018**

# Targets and therapies

**Retrospective (research)**: analysis of historic cohorts with known outcome to find prognostic biomarkers

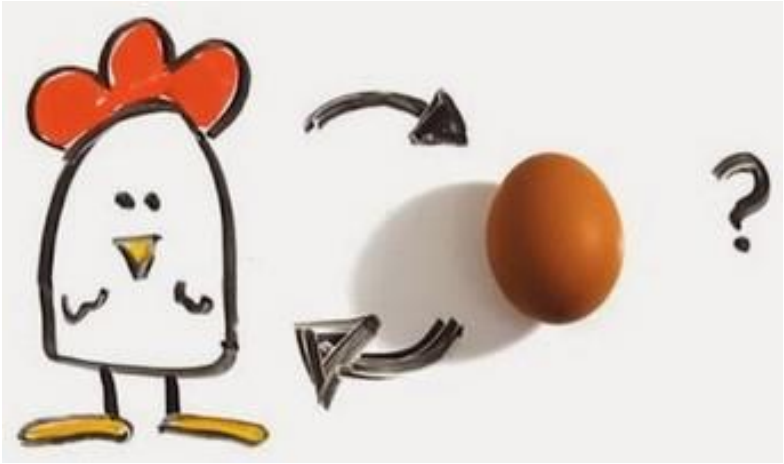
**Prospective (diagnostics)**: analysis of new patient's sample for risk stratification and targeted therapy

**Prospective (research):** advanced diagnostics to find targets for individualized treatment

# Targets and therapies

- To develop targeted therapies, we need to know what the targets are

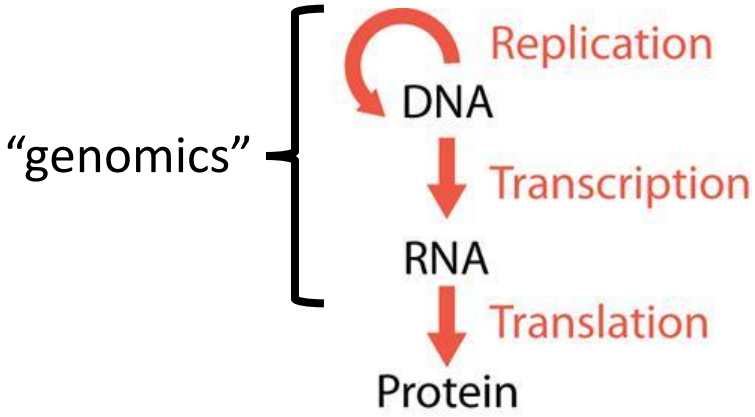
- To increase the success rate of clinical trials with specific inhibitors, we need to know which patients could benefit

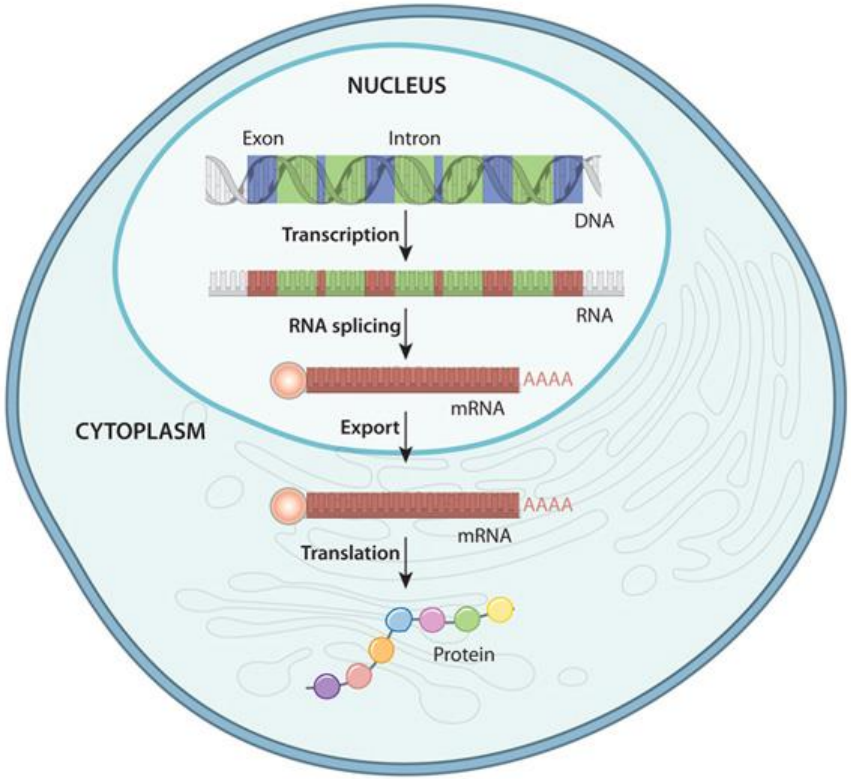


# I hope you will learn …

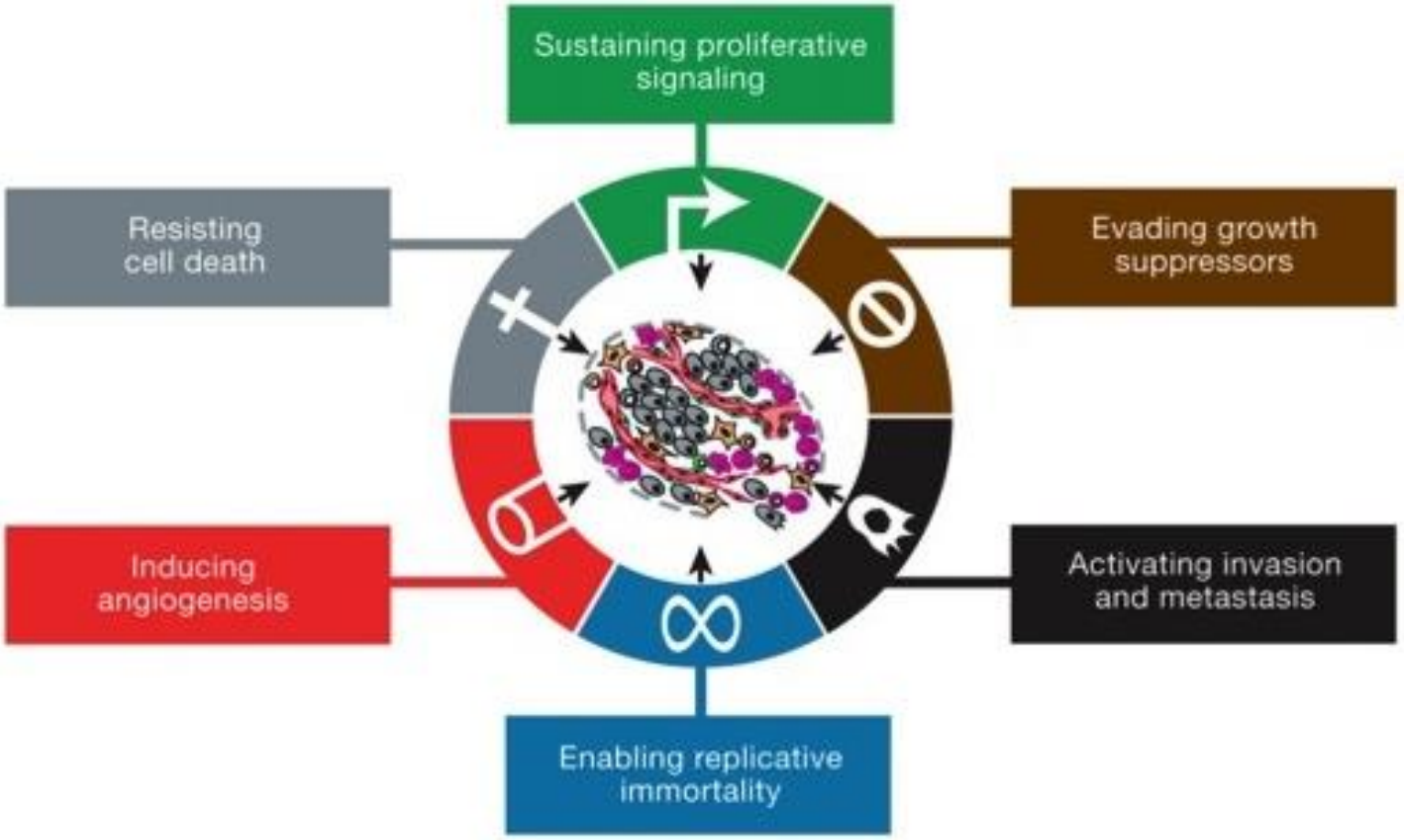
- which genetic alterations contribute to cancer
- how we detect genetic alterations
- how we interpret genetic alterations
- how we prioritize genetic alterations
- how we use this for personalized medicine

# The Central Dogma



# Cancer: a cell out of control

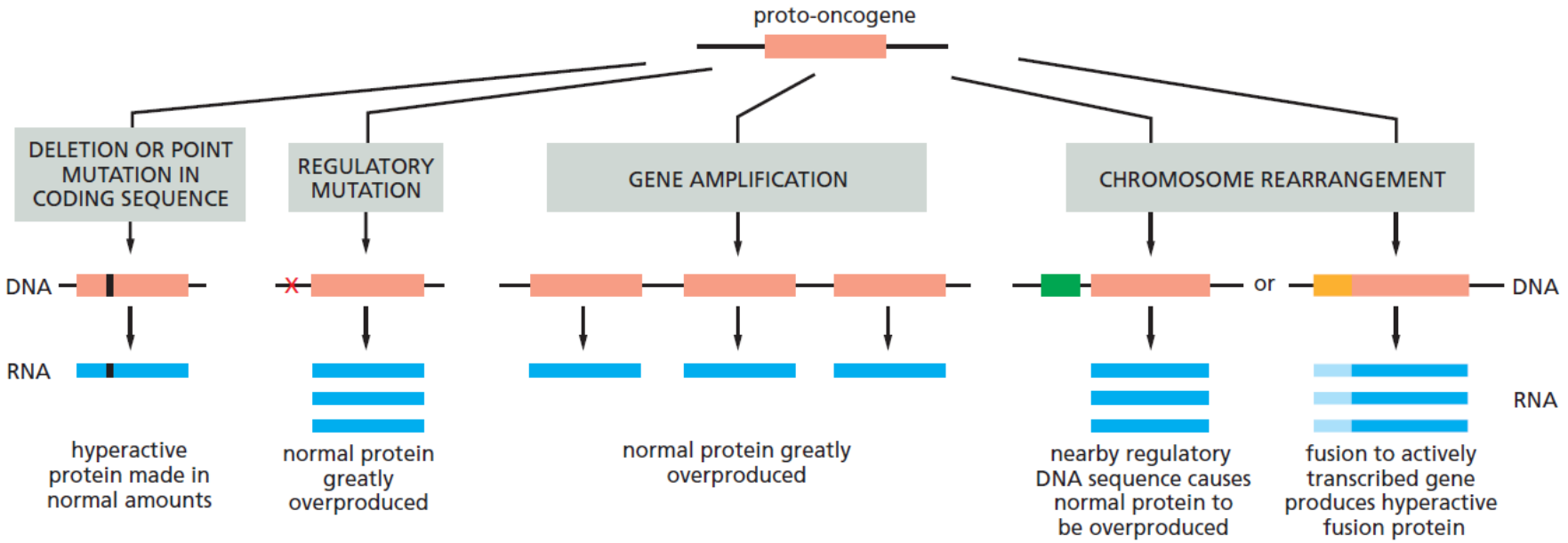



Hanahan D, Weinberg RA (January 2000). "The hallmarks of cancer". Cell. **100** (1): 57–70

# Cancer genes

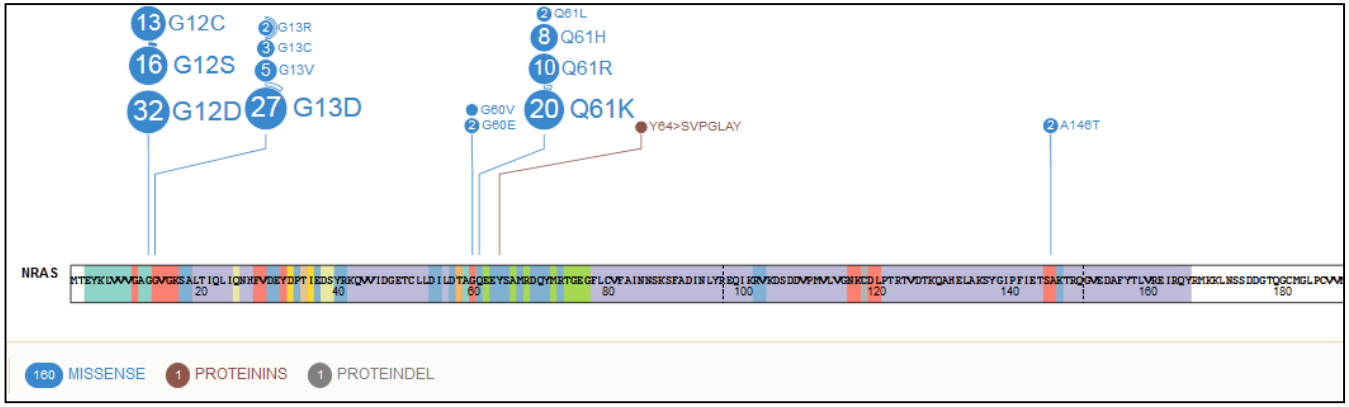
# Activation of proto-oncogenes

*activating mutation, overexpression, amplification, translocation, fusion*

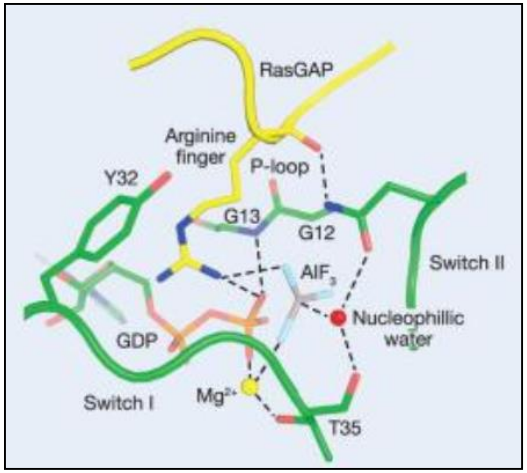


# Example oncogene: *RAS* mutation

**DNA mutation hotspots:** hitting amino acids in the active site



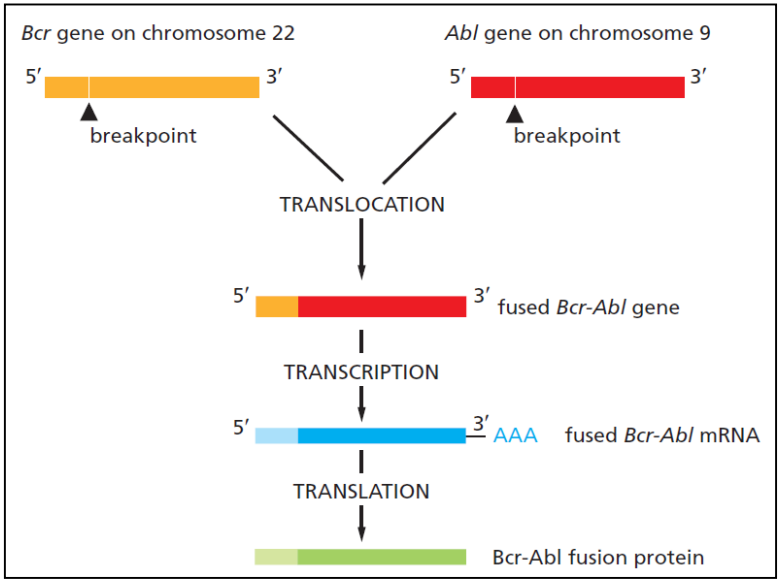

**Effect on protein:**
impaired ability of RAS mutants to hydrolyse GTP
➔ permanently “on”

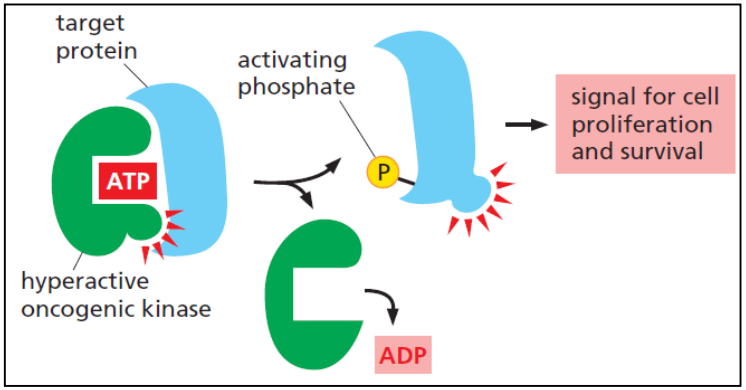


# Example oncogene: *BCR-ABL1*

**Chromosome translocation:**
*ABL1* kinase domain fused to *BCR*

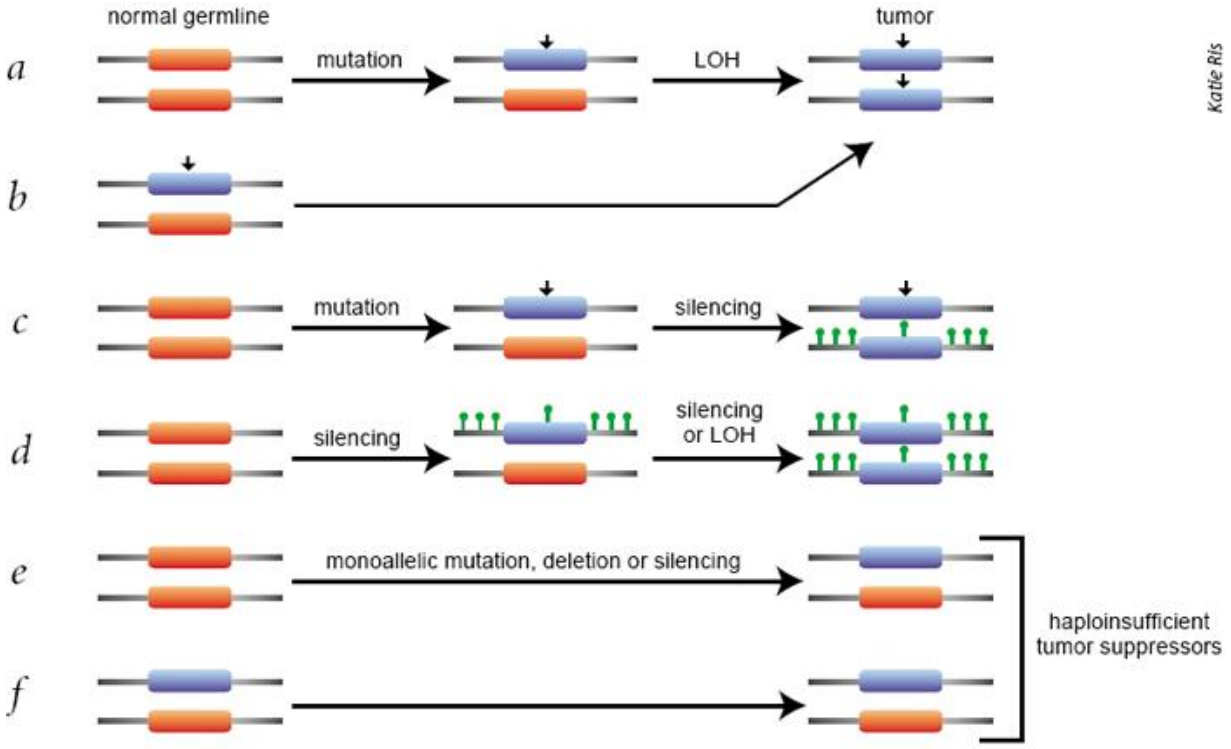
**Effect on protein:**
hyperactive oncogenic kinase



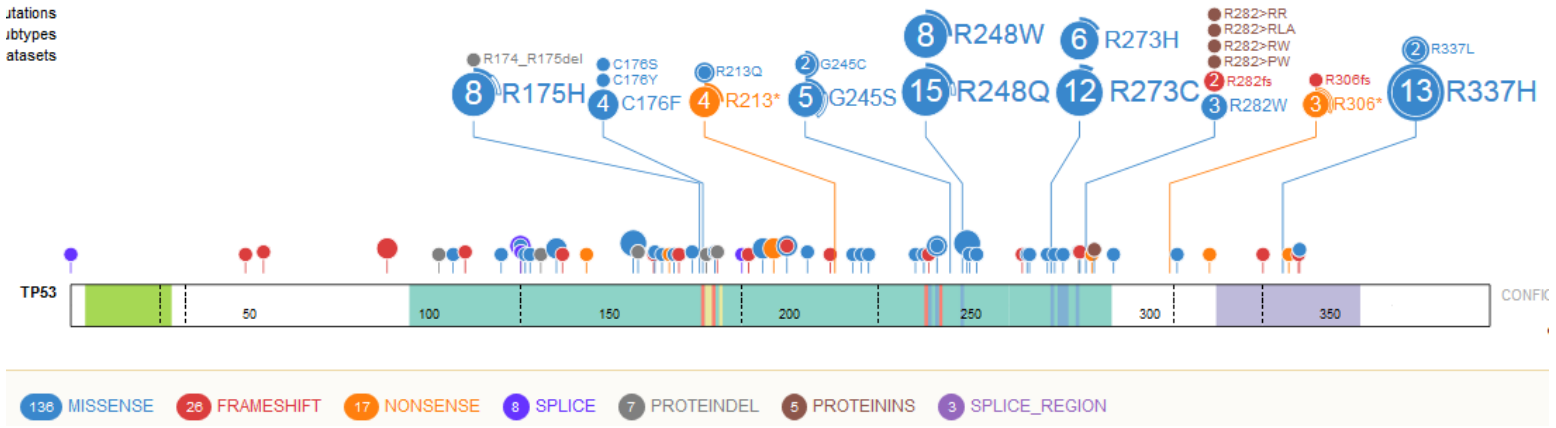
# Inactivation of tumor suppressor genes

*inactivating mutation, deletion, promoter silencing*



# Example: TP53 mutation

**DNA mutation:** inactivating and truncating mutations

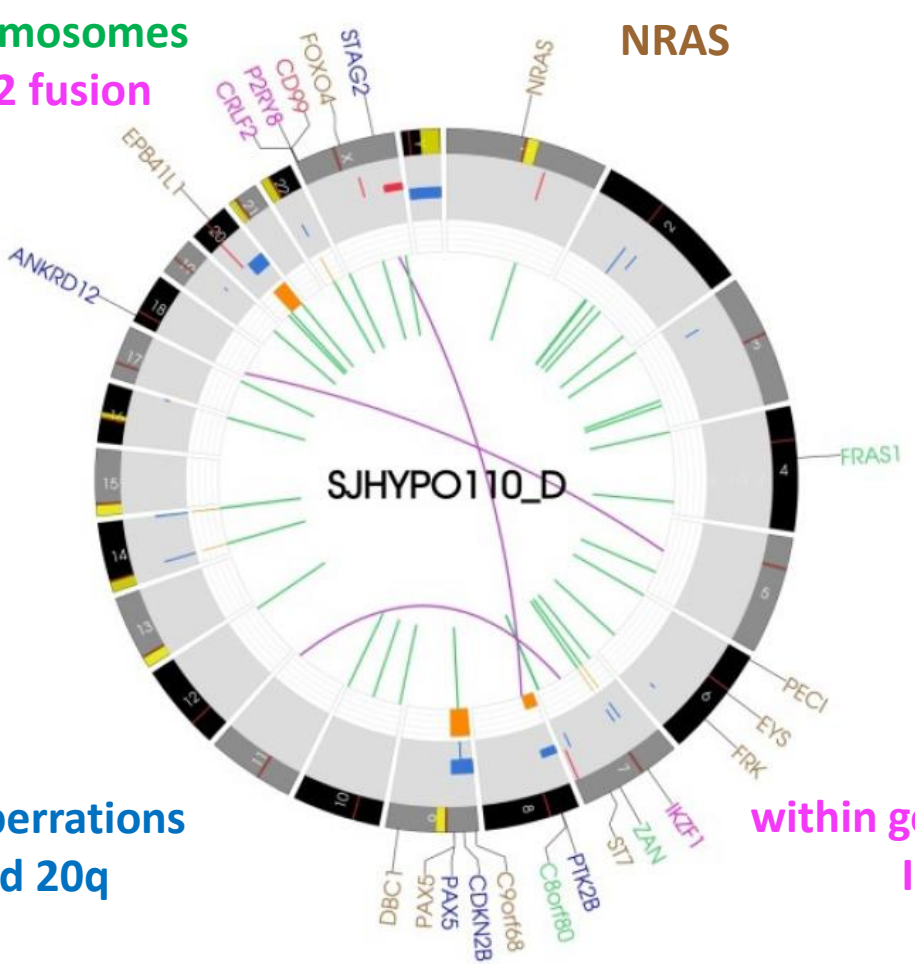



**Effect on protein:**
no/impaired DNA binding
➔ loss of function

# Cancer: accumulation of DNA changes

**rearrangements within and between chromosomes**
**P2RY8-CRLF2 fusion**

**mutations**
**NRAS**




Mutations
- Splice
- Exon
- UTR
- Intron
- Nonsense
- Missense
- Silent
- Protein Deletion
- Protein Insertion
- Frameshift

SV
- Gene
- Intra
- In frame gene fusion
- Interchromosomal

CNV
- Gene
- Loss
- Fusion

LOH
- LOH

**copy number aberrations**
**loss of 9p and 20q**

**within gene deletion**
**IKZF1**

ALL genome represented in a ‘circos plot’

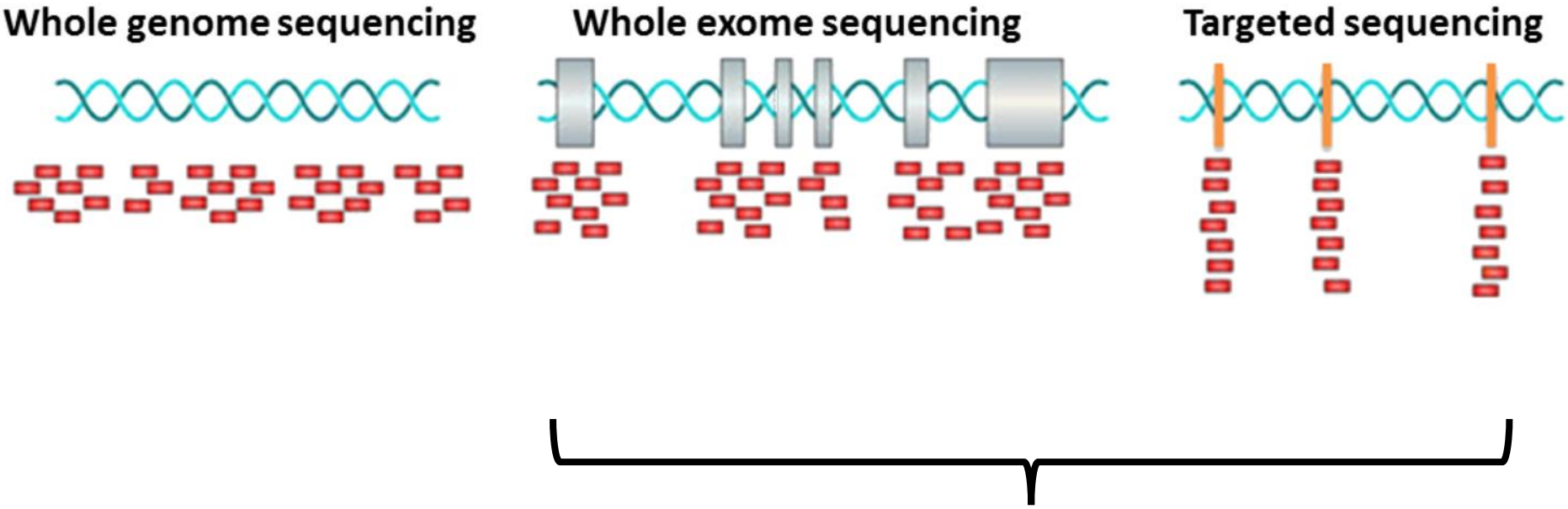
Roberts et al. NEJM 2014

# DNA changes define tumor characteristics

- Response to therapy
  - *ETV6-RUNX1* translocation sensitive to standard chemotherapy
  - Glucocorticoid resistance in RAS-mutated acute lymphoblastic leukemia
- Risk of relapse
  - *IKZF1* deletion associated with increased relapse risk in acute lymphoblastic leukemia
- Sensitivity for specific drugs
  - *BCR-ABL1* fusion sensitive to tyrosine kinase inhibitor imatinib

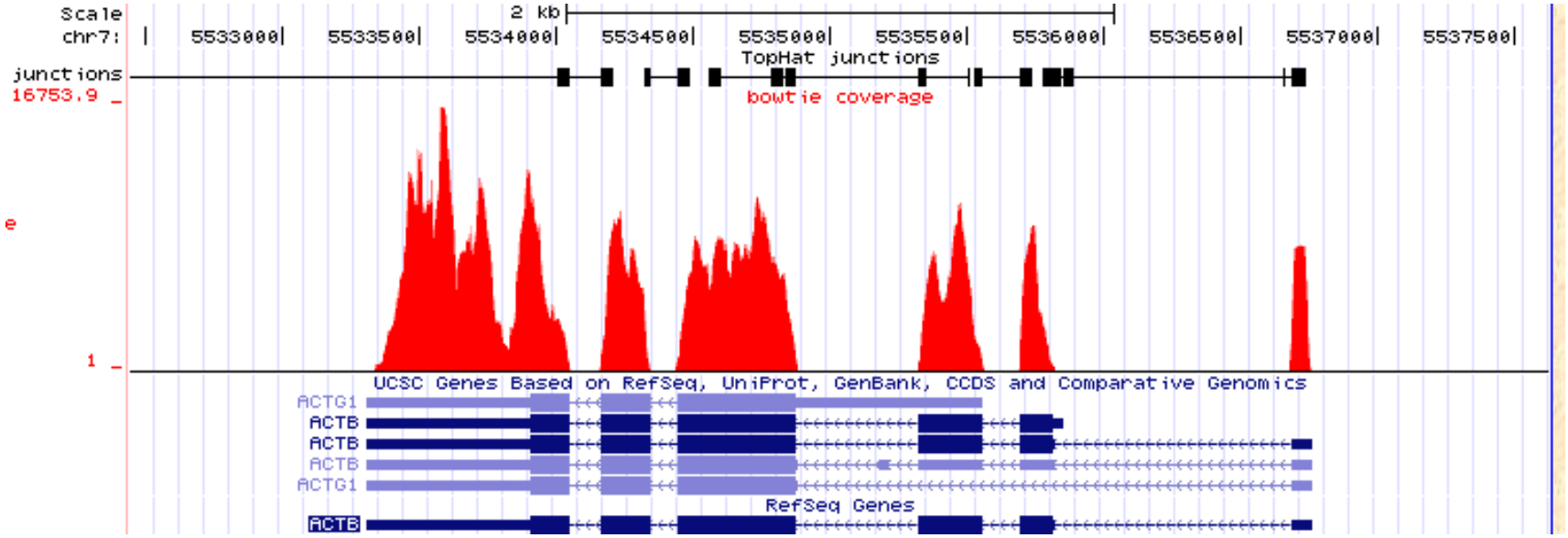
# Detecting alterations

# Next generation sequencing: DNA




capture the DNA of interest:
reduce the number of bases to sequence

# Next generation sequencing: RNA




starting from RNA: only the transcribed regions (exons) are covered

# Terminology

- **read**: sequence from next-generation sequencing machine
    - nucleotides (25-25,000 bases) with quality scores
- **alignment**: mapping read to reference genome
    - mismatches allowed; unmapped reads go to dustbin
    - reference bias: variant read has already 1 mismatch, so reference read has advantage

- **coverage**: proportion of targeted nucleotides covered by reads
- **depth**: number of reads covering a specific position

- **variant**: common name for mutation and polymorphism
    - single nucleotide variant (SNV), small insertion/deletion (indel)
    - structural variant (SV)
    - copy number variant (CNV)

# Viewing raw sequencing data


height: depth
color: SNV

grey: ref base

color: mismatch

lighter color:
low quality

color: reference
sequence (hg19)
G, A, T, C

single nucleotide variant

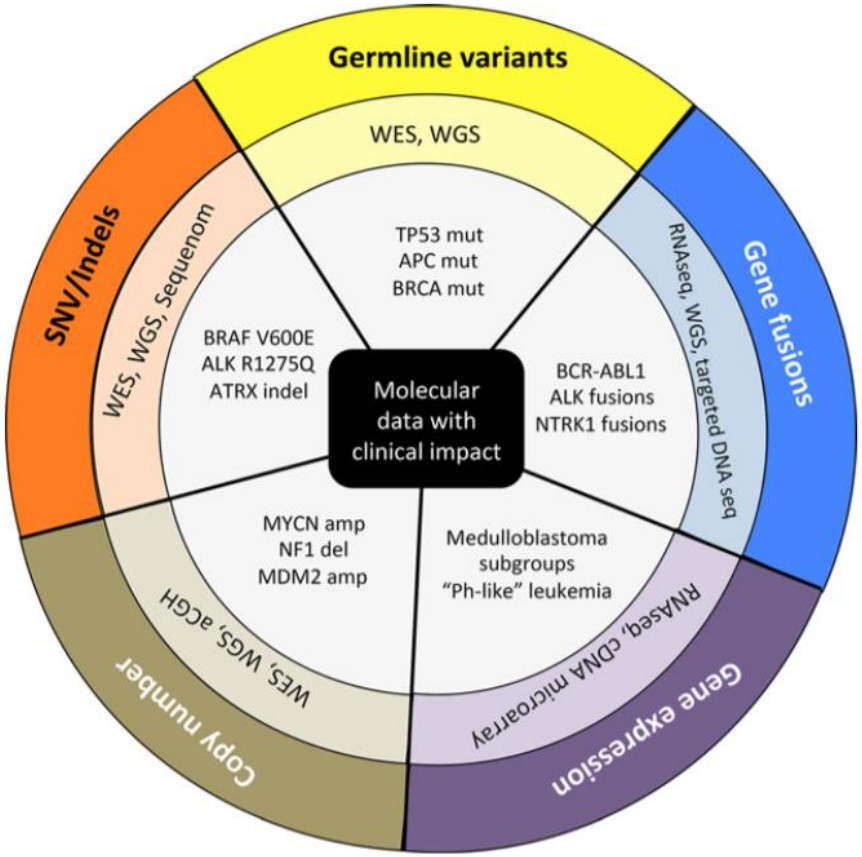
# Somatic versus germline variants

- **germline variants:** originate in germ cells
  - single nucleotide polymorphisms (SNV, indel)
  - small copy number variants (CNV)

- **somatic variants**: originate in tumor cells
  - SNV/indel, larger copy number alterations, rearrangements
  - tumor – normal (remission/blood) = somatic variants

- some germline variants cause cancer susceptibility

# Molecular data with clinical impact




different types of alterations

- SNV/indels (germline)
- copy number alterations
- rearrangements including gene fusion
- gene expression changes

require different detection methods

Mody et al. Pediatric Blood Cancer 2017; 64:e26288

# Detection of alterations

| Aberration | Variant type | Example leukemia | Whole Genome Sequencing | Whole Exome Sequencing | RNA Sequencing |
|---|---|---|---|---|---|
| translocation | change in position | *BCR-ABL1* | | | |
| expression | overexpression | *BCR-ABL1-like* | | | |
| aneuploidy | change in amount | high hyperdiploid (51-65 chromosomes) | | | |
| amplification | change in amount | RUNX1 | | | |
| deletion | change in amount | *IKZF1* deletion | | | |
| mutation/ indel | change in sequence | *JAK2* mutation | | | |
| cost estimate | | | | | |

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| mutation/ indel | change in sequence | *JAK2* mutation | V | V coding | V expressed |
| cost estimate | | | 100x @ €3000 | 100x @ €1000 | $50*10^6$ @ €500 |

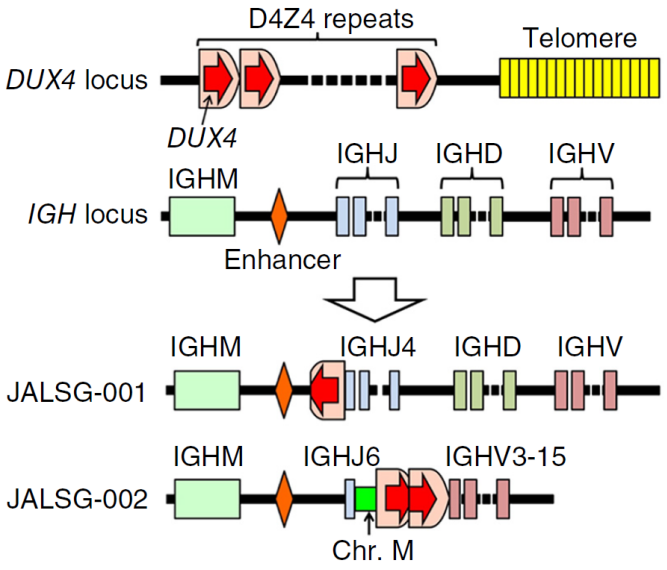
# Data quality and analysis

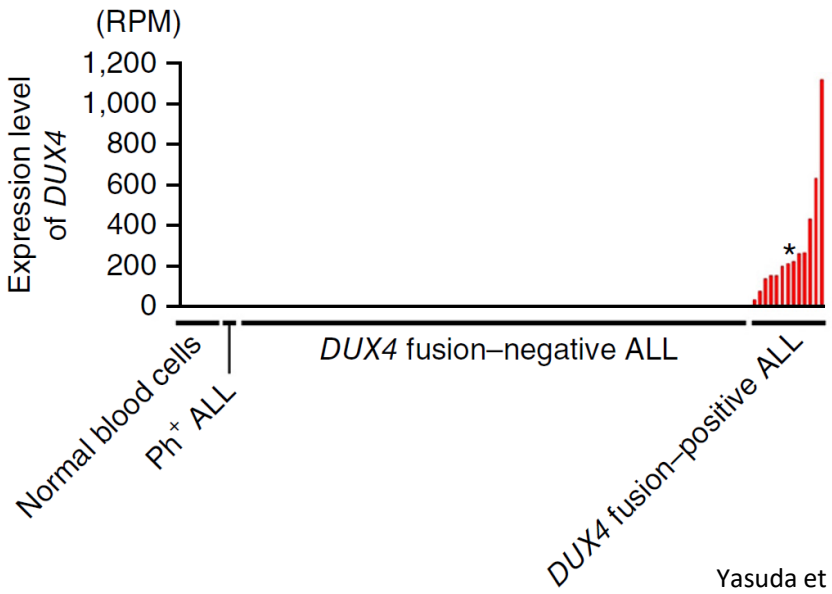
- If you find the aberration, how sure are you that it is present?
  - how many reads in total, how many alternative alleles? clonal or subclonal?
  - how many software methods detect the variant?
  - possible sequencing error, mapping error, low quality?

- If you don't find the aberration, how sure are you that it is absent?
  - sufficient coverage? hard to sequence, hard to map?
  - analysis method suitable to detect copy number and structural variants?
  - lost in filtering steps?

# Example leukemia: *DUX4* rearrangement

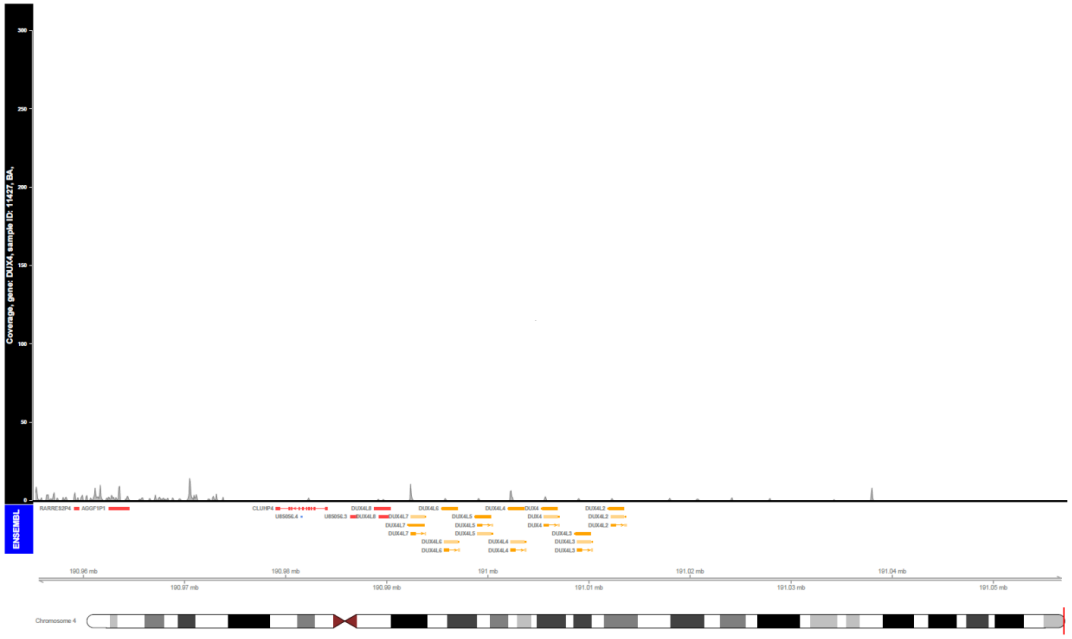
- 2012: new subtype discovered by gene expression profiling using arrays




Yasuda et al. 2016

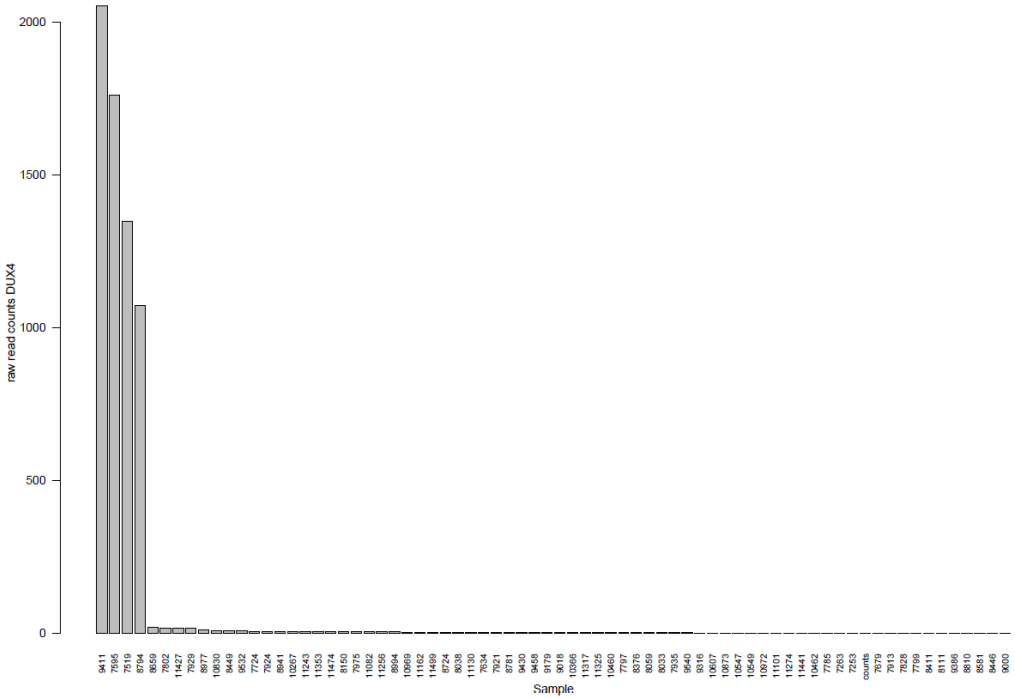
# Example leukemia: No *DUX4* expression?




Genome contains 3 regions with 100s of copies of *DUX4*

Aligner is confused and treats *DUX4* reads as bad quality reads ➔ dustbin

# Example leukemia: *DUX4* expression!




Solution: mask all but one *DUX4* gene in the reference genome

Now we detect 4 cases with high *DUX4* expression

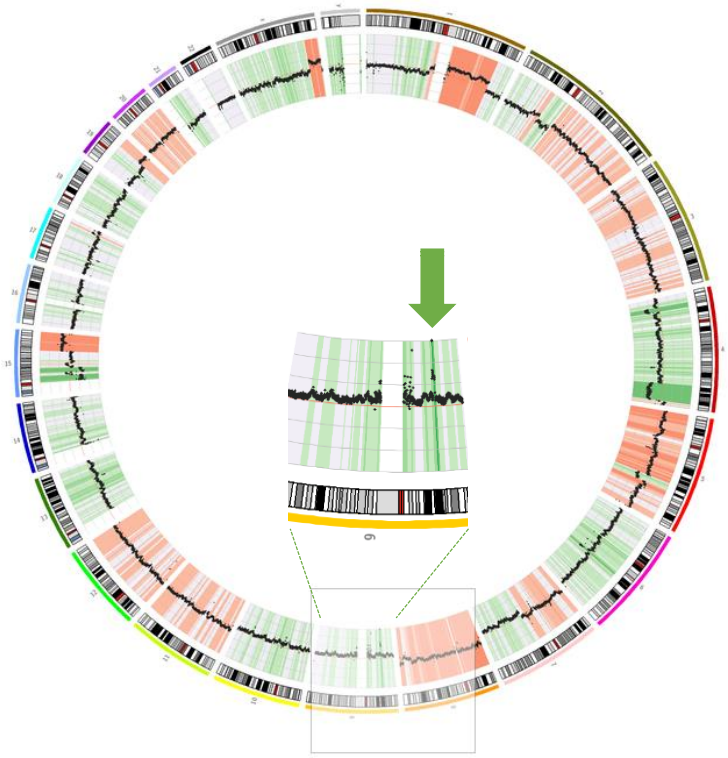
Inspection of sequence reads showed genomic insertion sites

# Interpreting alterations

# Somatic variant filtering

- Set thresholds for variant detection
  - minimal depth, minimal number of variant alleles, minimal clonality (variant allele frequency)
- Combine data from several sources (DNA and RNA)
  - variant allele expressed? amplified and overexpressed?
- Evaluate the effect of the variant on protein activity
  - amino acid change (missense), stop codon (nonsense), frameshift

# Copy number profile



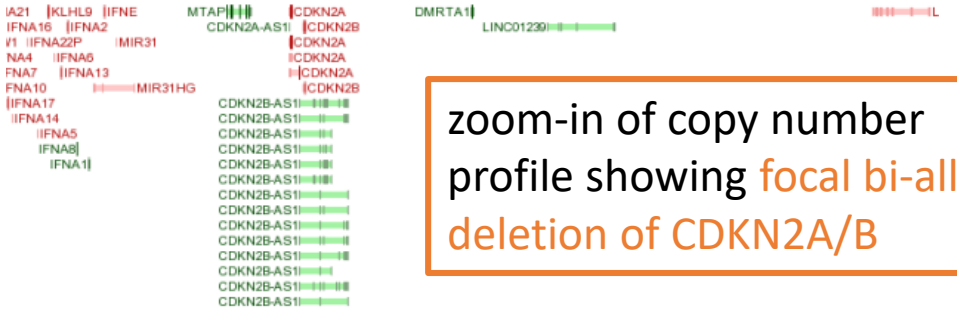
circos plot with tracks for

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

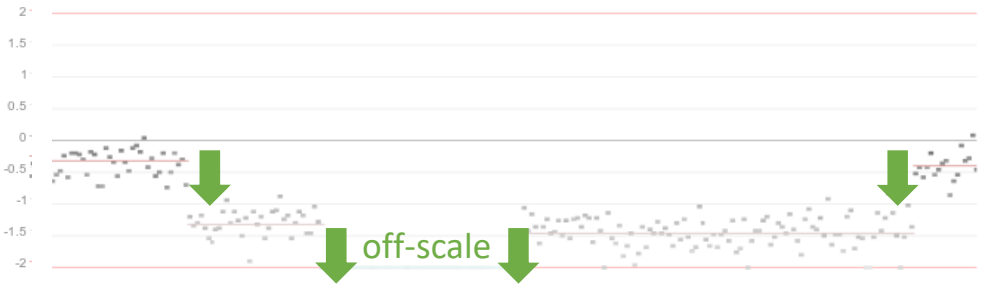
copy number list showing CDKN2A/B loss

gains (red) | losses (green)

| link | Gene | logfold | Info | link | Gene | logfold | Info |
|---|---|---|---|---|---|---|---|
| View | KIR2DL3 | 1.2065 | chr19:55250000-55259999 (9999) | View | CDKN2B | -5.1686 | chr9:21940000-22459999 (519999) |
| View | KIR2DL1 | 1.2065 | chr19:55290000-55299999 (9999) | View | CDKN2A | -5.1686 | chr9:21940000-22459999 (519999) |
| View | IGF1R | 0.8115 | chr15:99270000-100269999 (999999) | View | VEGFC | -1.4109 | chr4:177560000-178559999 (999999) |
| View | NTRK1 | 0.809 | chr1:156140000-157139999 (999999) | View | PDGFC | -1.2571 | chr4:156880000-157879999 (999999) |
| View | MUC1 | 0.809 | chr1:155140000-156139999 (999999) | View | FBXW7 | -1.2376 | chr4:153120000-153409999 (289999) |
| View | IL6R | 0.809 | chr1:154140000-155139999 (999999) | View | RAD51 | -1.1628 | chr15:40340000-41339999 (999999) |
| View | FGFR1 | 0.7963 | chr8:38000000-38789999 (789999) | View | SPRED1 | -1.1628 | chr15:38340000-39339999 (999999) |




zoom-in of copy number profile showing focal bi-allelic deletion of CDKN2A/B



# Somatic (=tumor) mutations/indels


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 | 11106664 | G | T | 0.45 | 0 | MASP2 | p.D120E | Info | |
| view | chr1 | 35370138 | C | A | 0.97 | 0 | DLGAP3 | p.E282D | Info | |
| view | chr1 | 45252269 | C | T | 0.96 | 0 | BEST4 | p.G116S | Info | |
| view | chr1 | 65895641 | AGT | A | 0.45 | 43 | LEPROT | p.64_64del | Info | |
| view | chr1 | 85725078 | T | C | 0.48 | 52 | C1orf52 | p.I80V | Info | |
| view | chr1 | 115256529 | G | T | 0.56 | 93 | NRAS | p.Q61K | Info | C A |
| view | chr1 | 158449823 | T | C | 0.24 | 0 | OR10R2 | p.F53L | Info | |
| view | chr1 | 182853858 | G | C | 0.17 | 489 | DHX9 | p.L1124F | Info | |
| view | chr1 | 207245657 | G | A | 0.20 | 3 | PFKFB2 | p.R487Q | Info | |
| view | chr10 | 55945006 | G | C | 0.51 | 3 | PCDH15 | p.L443V | Info | |
| view | chr11 | 407099 | G | C | 0.19 | 92 | SIGIRR | p.D230E | Info | |
| view | chr11 | 5068253 | C | T | 0.31 | 0 | OR52J3 | p.R167C | Info | |
| view | chr11 | 18955646 | C | A | 0.18 | 0 | MRGPRX1 | p.G229C | Info | |
| view | chr11 | 47176706 | AT | A | 0.30 | 3 | C11orf49 | | Info | |
| view | chr11 | 48285651 | >8bases | C | 0.16 | 0 | OR4X1 | p.81_86del | Info | C |
| view | chr11 | 56019854 | A | AT | 0.30 | 0 | OR5T3 | p.F61fs | Info | |
| view | chr11 | 64888273 | G | A | 0.28 | 1988 | FAU | p.A59V | Info | |
| view | chr11 | 76507249 | A | G | 0.31 | 80 | TSKU | p.H197R | Info | |
| view | chr11 | 85418517 | A | G | 0.29 | 31 | SYTL2 | p.L687P | Info | |
| view | chr11 | 89431855 | AT | A | 0.29 | 0 | FOLH1B | NA | Info | |
| view | chr11 | 89450732 | A | G | 0.29 | 0 | TRIM77 | p.D349G | Info | |
| view | chr11 | 107462678 | TGCTGGG | T | 0.17 | 0 | AP000889.3 | p.66_68del | Info | |
| view | chr11 | 108183179 | TAGC | T | 0.24 | 5 | ATM | p.1988_1988del | Info | C A |

etc.

C: Cosmic Mutation
A : Actionable Gene (accoring to grp.ither.ither_genelist_v2018)
VAF 0.10: VAF <=0.1

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

NRAS located on 1p in region CN gain: 2/3 mutated copies x 80% tumor cells ➔ predicted VAF 0.53, observed VAF 0.56

VarRNA: indication of expression level of variant allele

NRAS variant allele is expressed

OncoKB database has information on NRAS mutation:

NRAS Q61K mutation is known oncogenic

All Variants

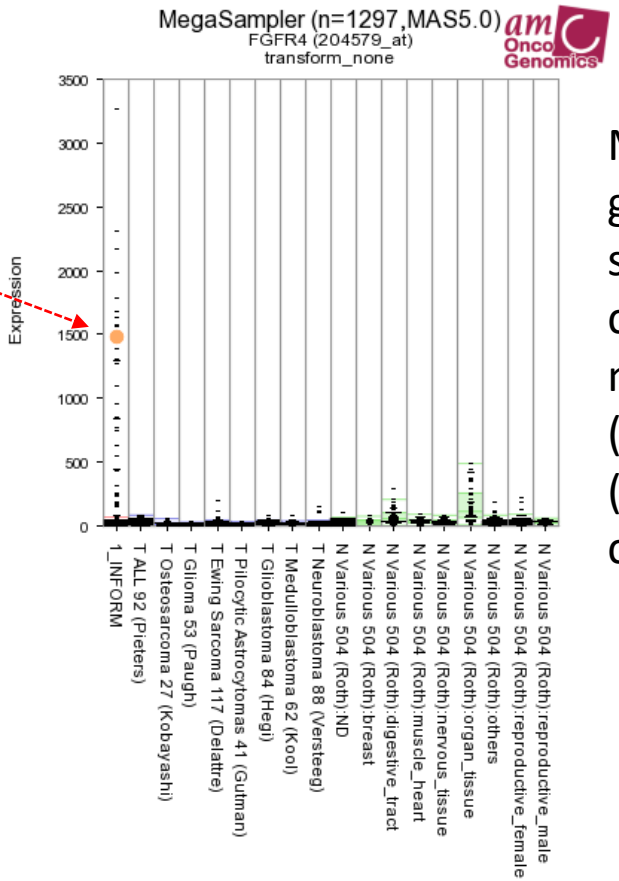
| Gene | Alteration | Oncogenicity | Mutation Effect |
|---|---|---|---|
| NRAS | Q61K | Oncogenic | Gain-of-function |

# Gene expression

| 1GeneView | Gene | zscore | Detailed |
|---|---|---|---|
| 208694_at | PRKDC | 2.646 | Detailed |
| 201244_s_at | RAF1 | 2.371 | Detailed |
| 204579_at | FGFR4 | 2.350 | Detailed |
| 207145_at | MSTN | 2.200 | Detailed |
| 210220_at | FZD2 | 2.059 | Detailed |

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

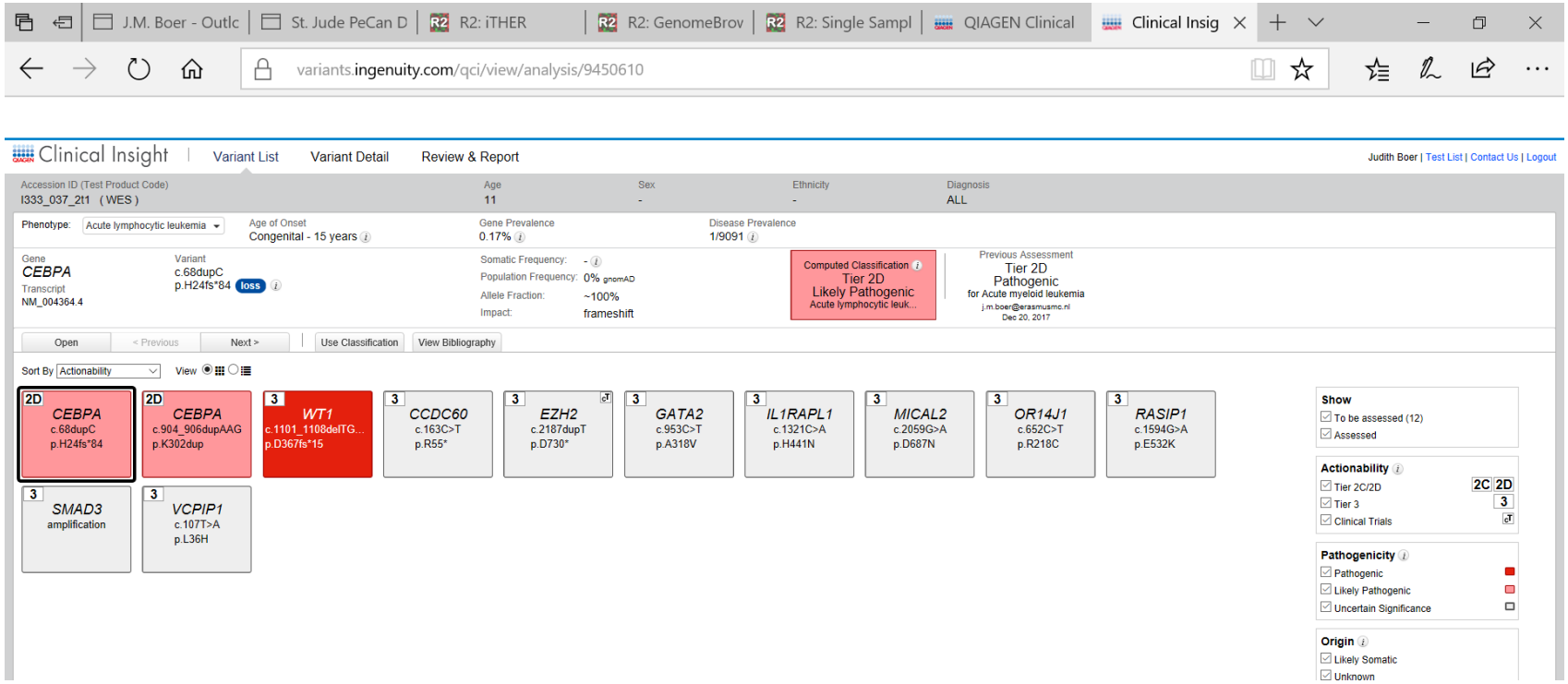
This RMS sample (orange dot) has high FGFR4 expression




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

# Qiagen Clinical Insight

## Pathogenicity & actionability



# Prioritizing alterations

for personalized medicine

# Prioritizing actionable events

**1. Is the event drugable?** **Yes**/**No**, but tumor-biologically relevant

**2. Is the event a genetic change?** **Yes**/**No**, for example expression change

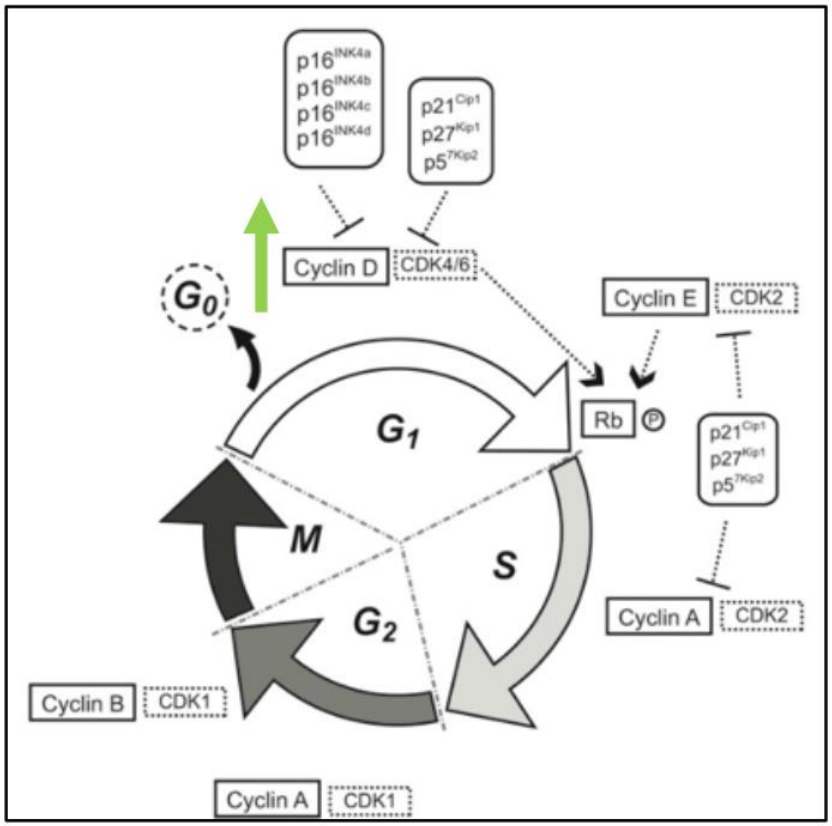
**3. Is the event a direct drug target?** **Yes**/**Pathway**/**No**, but susceptibility to inhibition of another gene

**4. What is the evidence level for the event to activate a driver gene or pathway?** **Confirmed**/**Presumed**/**Possible**

**5. Is the event specific for the tumor type?** **Yes**/**No**

# Direct and indirect drug targets

CDK4/6 inhibitors of the cell cycle:
CDKN2A/B indirect target
CDK4/6 direct target



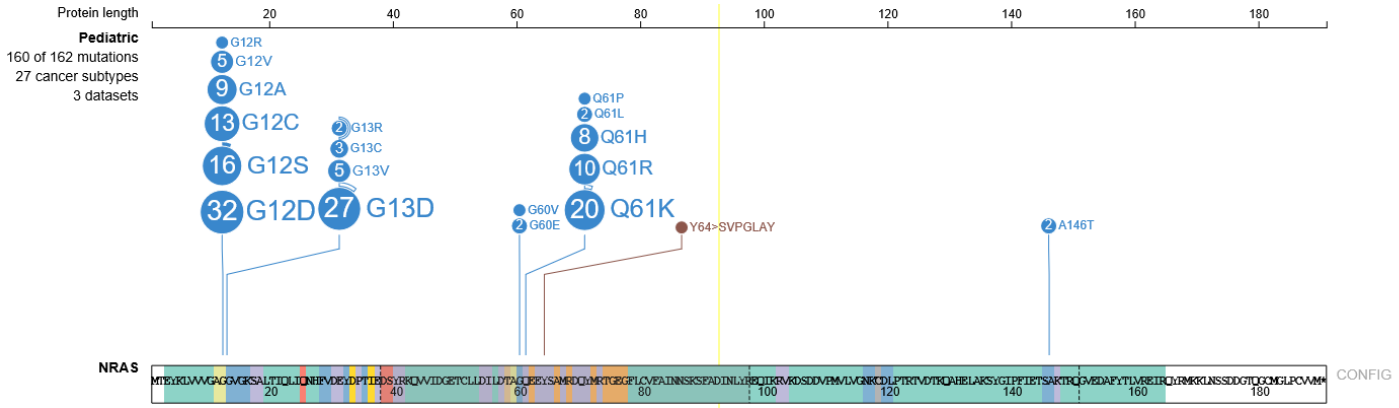

**Compound availability**

**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

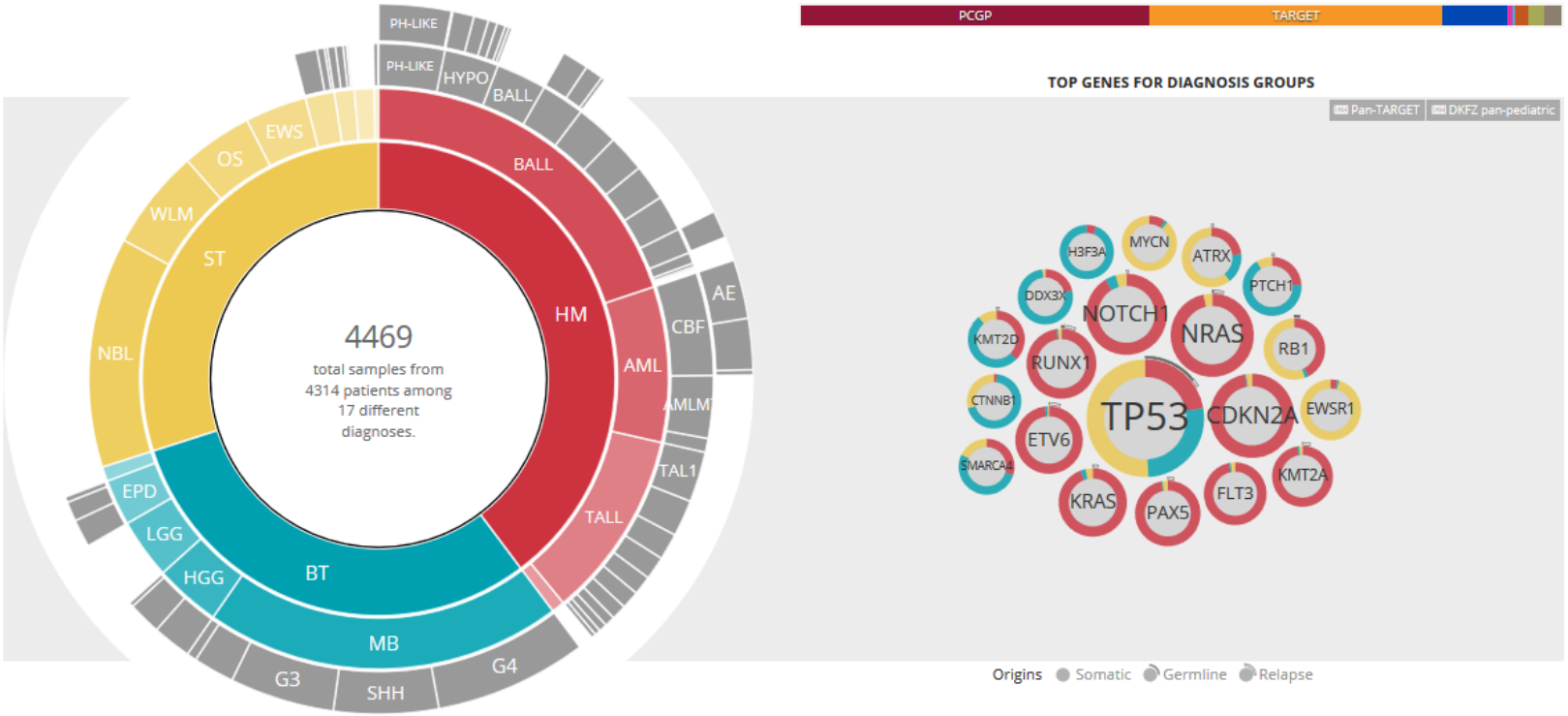
# Has the exact variant been observed before?

- Recurrent mutations in pediatric cancer: PeCan hosted by St. Jude
  - recurrent mutations/hotspots
  - protein domains



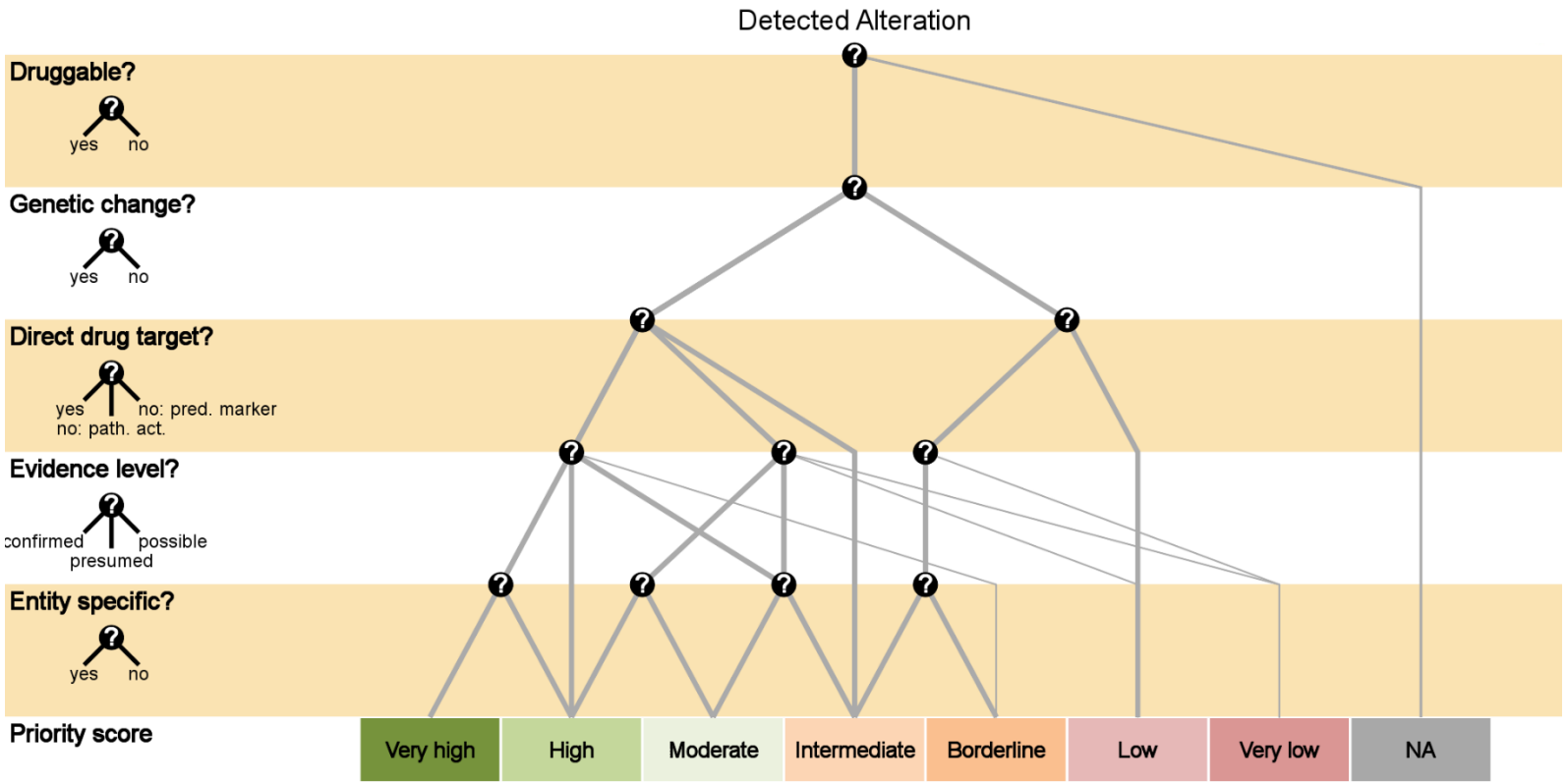

Source: Pediatric Cancer Database, St. Jude: pecan.stjude.org

# Is the target specific for the cancer subtype?

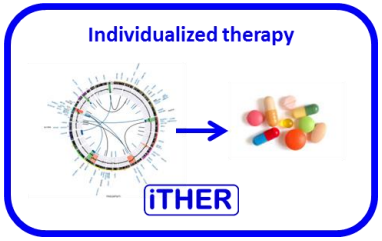



Source: Pediatric Cancer Database, St. Jude: pecan.stjude.org

# Target prioritization



# Personalized medicine

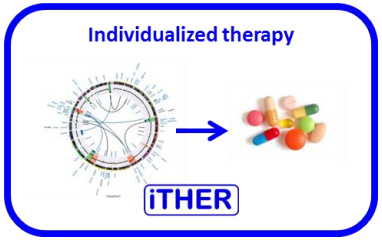



- use the identified variants to suggest treatment for individual patients for whom no regular treatment options remain
- use the genetic biomarkers to develop better early clinical trials
    - response may depend on presence of the genetic alteration

➔ individualized therapies for children with relapsed/refractory cancer (iTHER)

- Jan Molenaar, Michel Zwaan, Monique den Boer
- collaboration with INFORM

# iTHER study




- feasibility study:
  - DNA/RNA isolation, sequencing, detection of actionable lesions, advice for available compounds and clinical trials

- next-gen sequencing:
  - WES 150x depth tumor; 100x depth germline DNA ➔ SNV, indel
  - WGS 5x depth tumor; 2x depth germline DNA ➔ CNV
  - mRNA-seq: 60 million read-pairs; Affymetrix array ➔ fusions, expr

- 3-4 weeks from biopsy to advice in molecular tumor board

# Molecular Tumor Board

where it all comes together!

# Molecular Tumor Board

- Pediatric oncologist treating the patient
- Oncologist specialized in early clinical trials
- Genomics researcher specialized in the tumor type
- Bioinformatician specialized in data analysis
- Pathologist to comment on the biopsy
- Clinical geneticist to comment on germline variants
- Study coordinator

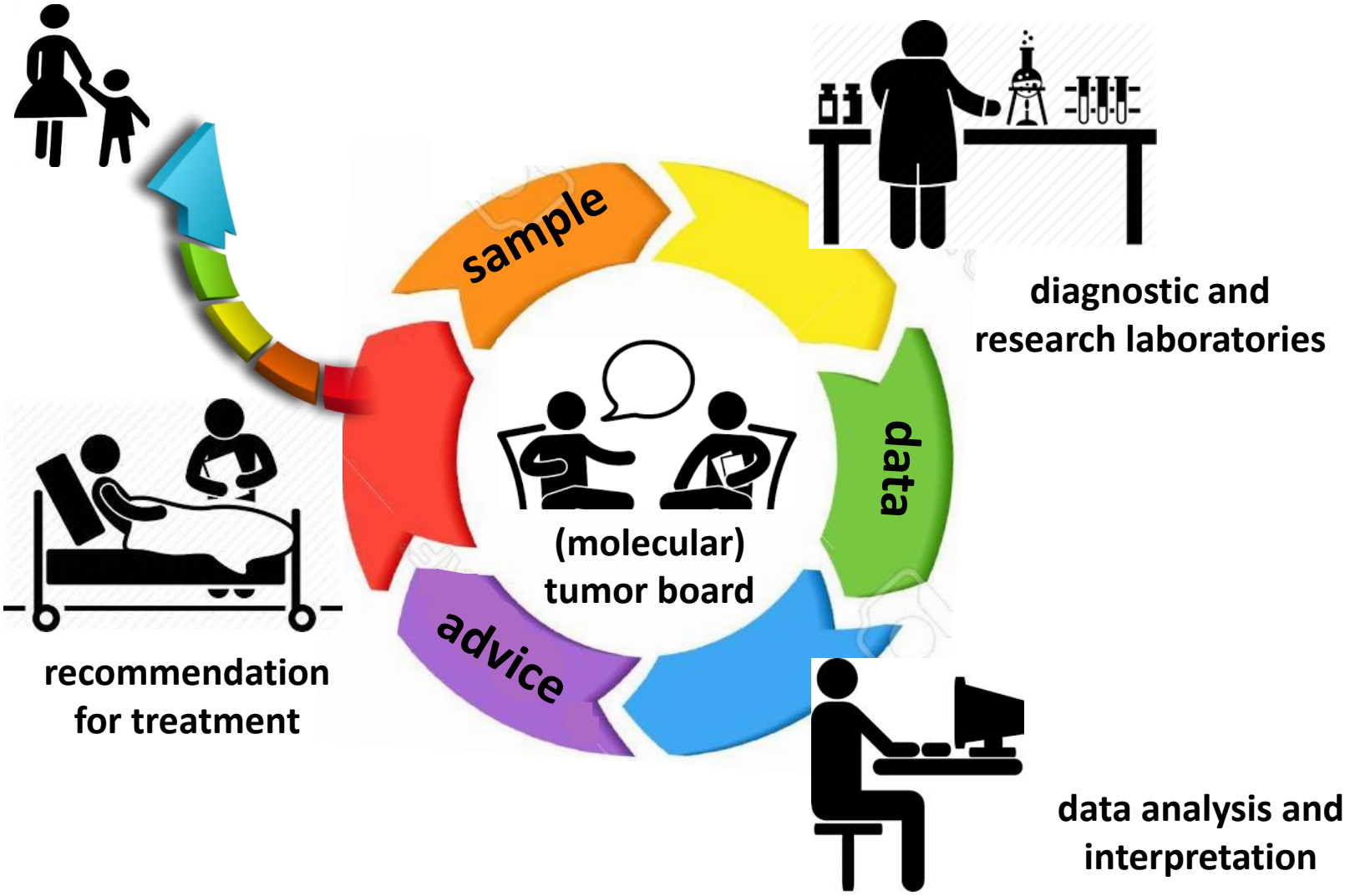
# How often are we successful?

- Availability of tissue: tumor and normal
  - leukemia relapse after stem cell transplant: germline of patient and donor
  - biopsy with sufficient tumor content, not too much necrosis
- Technical success rate
  - amount and quality of isolated DNA and RNA
- Identification of actionable lesions
  - variants scored intermediate/moderate/high/very high (~50%)
  - arms open in clinical trials, drug available for off label use (<25%)

Worst et al. Eur J Cancer 2016 – The INFORM pilot study

# I hope you learned …

- which genetic alterations contribute to cancer
  - activation of oncogenes, inactivation of tumor suppressors
- how we detect genetic alterations
  - DNA and RNA sequencing, (FISH, RT-PCR, microarrays)
- how we interpret genetic alterations
  - public data on variant types, frequencies, protein domains
- how we prioritize genetic alterations
  - decision tree to score the alterations as oncogenic driver
- how we use this for personalized medicine
  - matching genetic alterations with available trials/compounds


sample
data
advice
(molecular)
tumor board
diagnostic and
research laboratories
recommendation
for treatment
data analysis and
interpretation

## B-cell precursor leukemia program

- Alex Hoogkamer
- Marjolein Bakker
- Aurelie Boeree
- Femke Meijers
- Myrthe Vermeeren
- Femke Hormann
- Naomi Michels
- Mandy Smeets
- Iris van de Sandt
- Ilse Dingjan
- Cesca van de Ven
- Monique den Boer

## iTHER team NL

- Michel Zwaan
- Bianca Goemans
- Jan Molenaar
- Monique den Boer
- Willemijn Breunis
- Esther Hulleman
- Jan Koster
- Miriam Stumpf

# ITCC INTRODUCTORY COURSE IN PAEDIATRIC DRUG DEVELOPMENT

## CONNECTED TALKS & WORKSHOP TOMORROW:

Birgit Geoerger – Precision medicine trials

Jan Molenaar – Preclinical drug discovery

Fernando Carceller & Judith Boer –

WORKSHOP 5: Simulated Molecular Tumor Board

ITCC European consortium