Joni Van der Meulen - MDG Ghent University Hospital

molecular profiling of solid tumors and hematological malignancies using targeted sequencing

22nd annual BSMO meeting 2020
february 15th 2020

UZ GENT
UNIVERSITEIT GENT

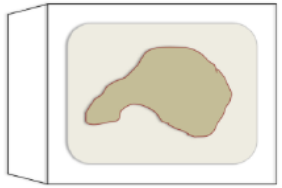
platform molecular diagnostics UZ Gent (MDG)

center for medical genetics (CMGG)

clinical biology

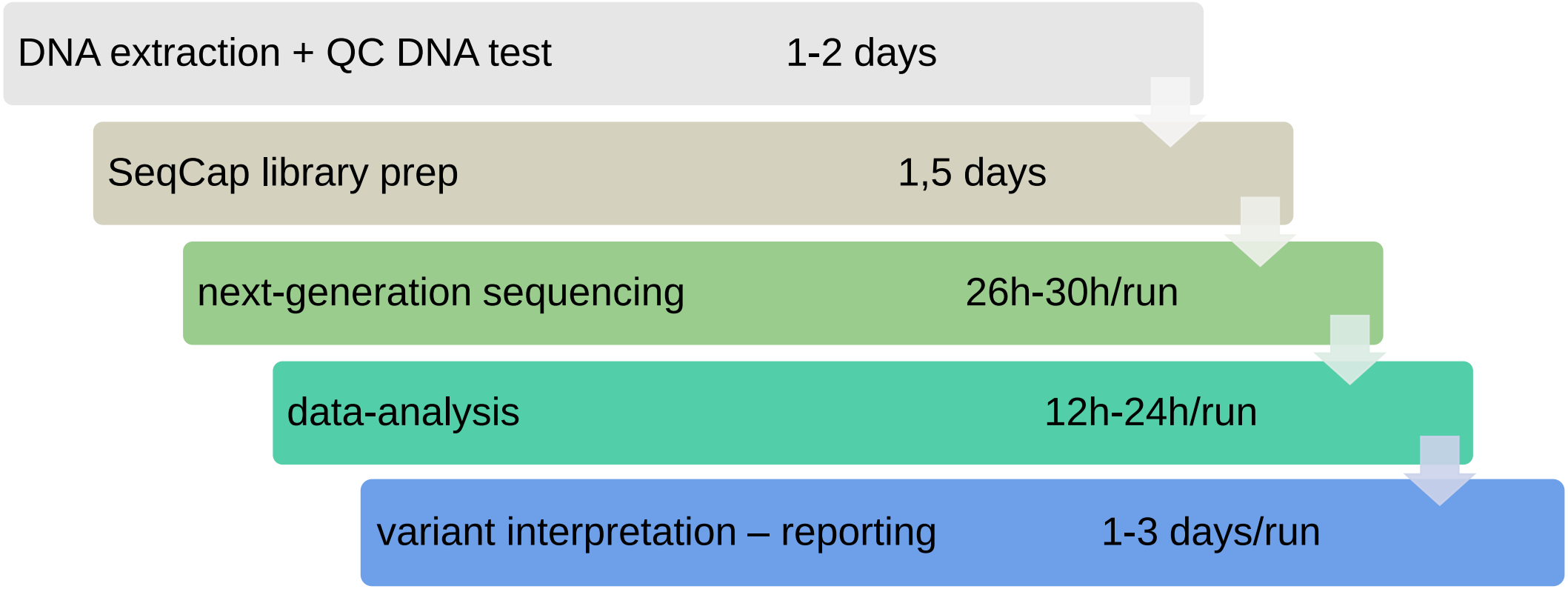
pathology

targeted next-generation sequencing (NGS) to define diagnosis, prognosis and prediction of therapy response

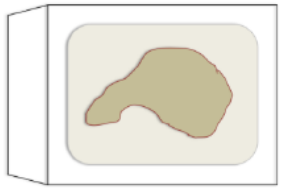


from request till report to the clinici variant interpretation/classification

next-generation sequencing workflow



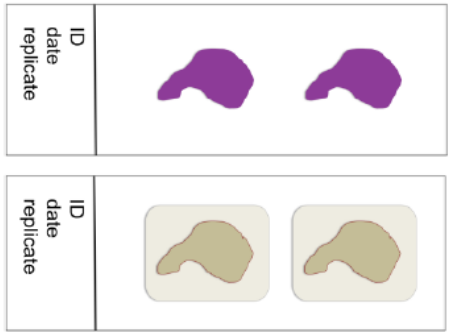
DNA extraction + QC DNA test (solid tumors)



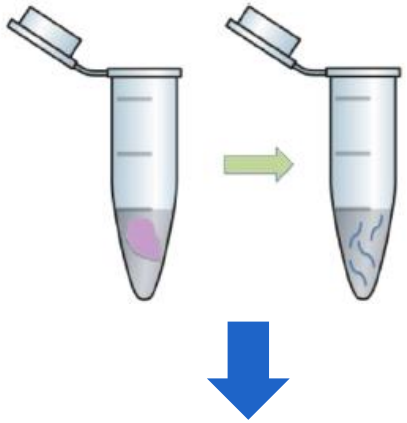
>10% tumor cells

time between biopsy-resection and fixation < 1h

fixation time: 6-72h in 10% neutral buffered formalin



1 H&E slide
5 slides of 10µM

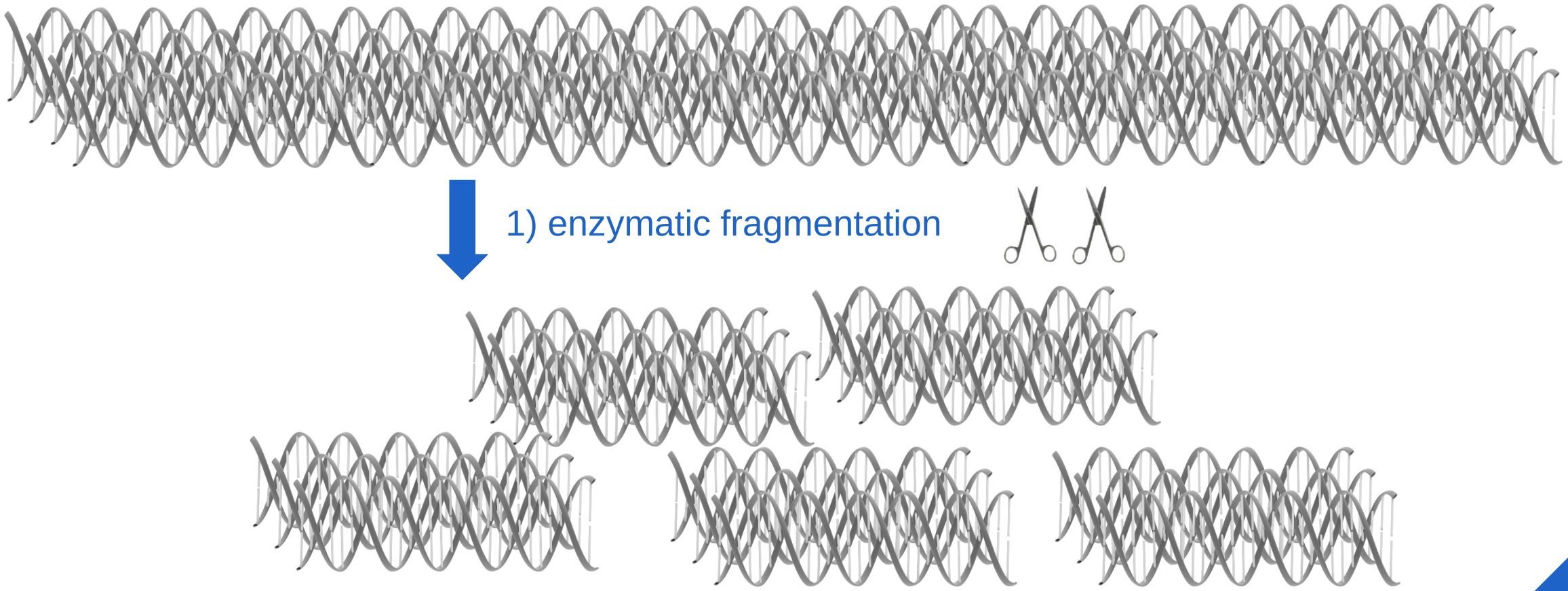


High-quality hgDNA

Damaged hgDNA

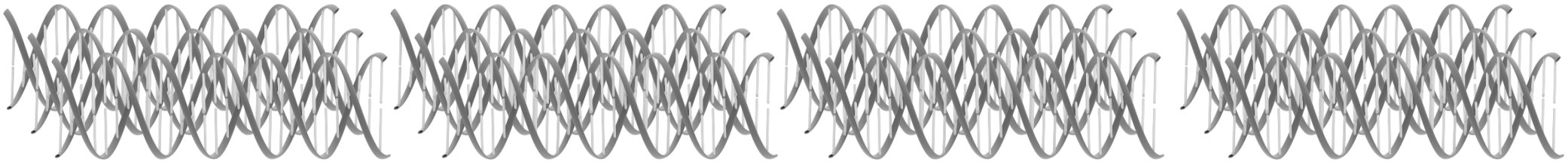
SeqCap library prep – capture-based NGS

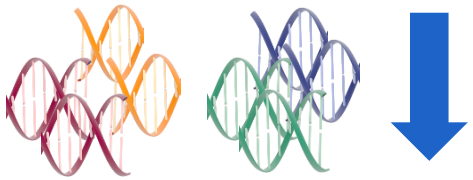
enzymatic fragmentation



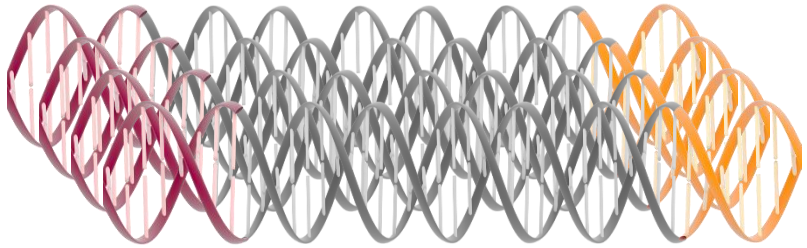
SeqCap library prep – capture-based NGS

ligation of adapters with unique dual indexes (UDI)

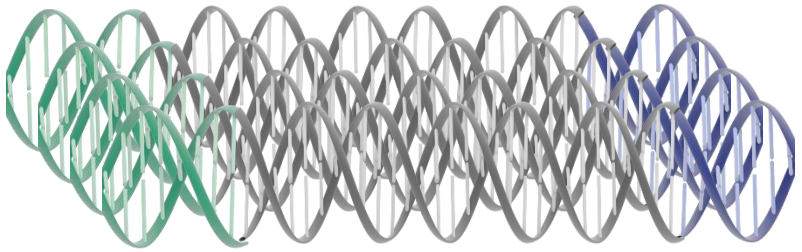




2) ligation of adapters with unique dual indexes (UDI)
*adapters for binding to the flowcell and sequencing of primer sites
* unique dual indexes ≠ for each patient sample



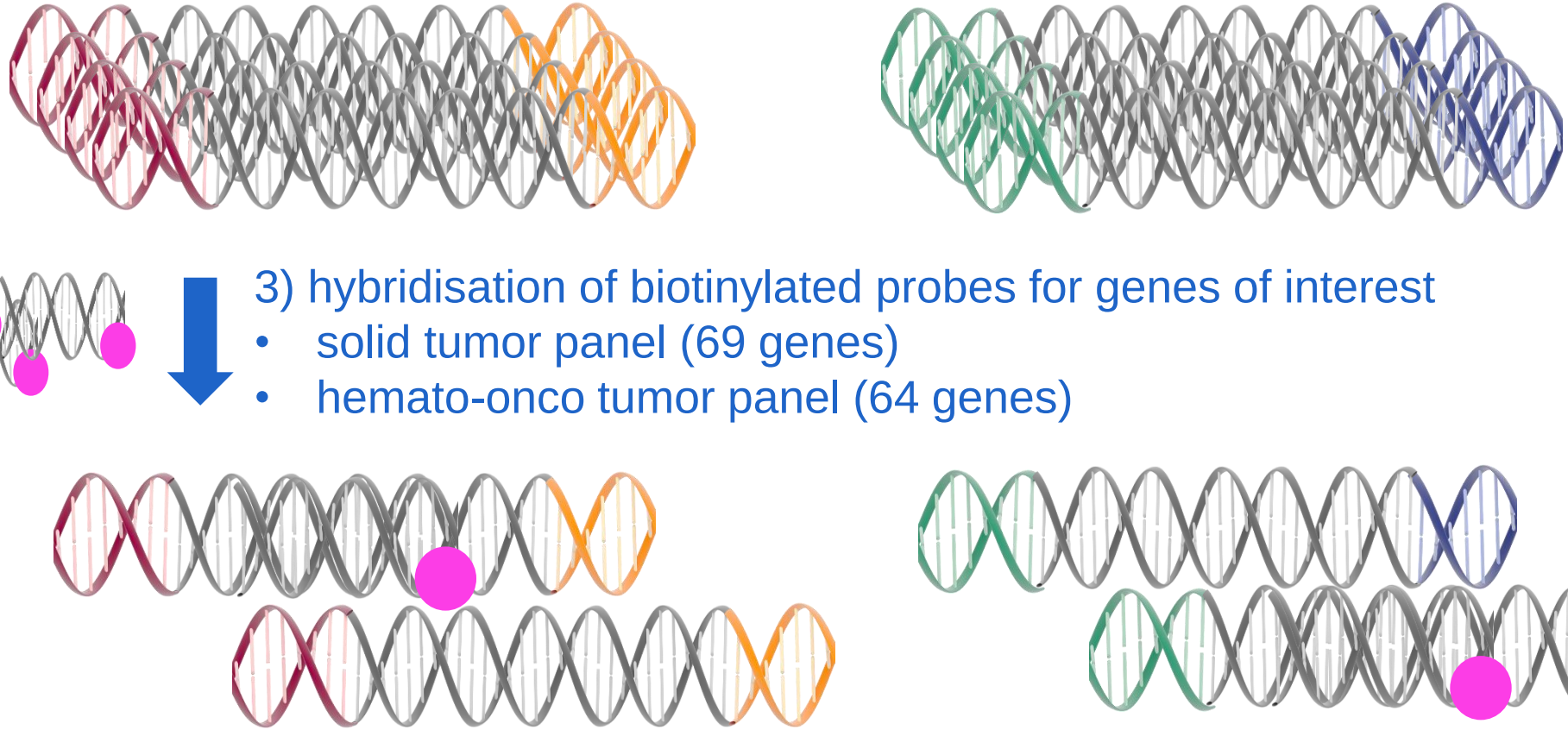
patient sample 1



patient sample 2

SeqCap library prep – capture-based NGS

probe hybridisation



3) hybridisation of biotinylated probes for genes of interest

- solid tumor panel (69 genes)
- hemato-onco tumor panel (64 genes)

SeqCap library prep – capture-based NGS

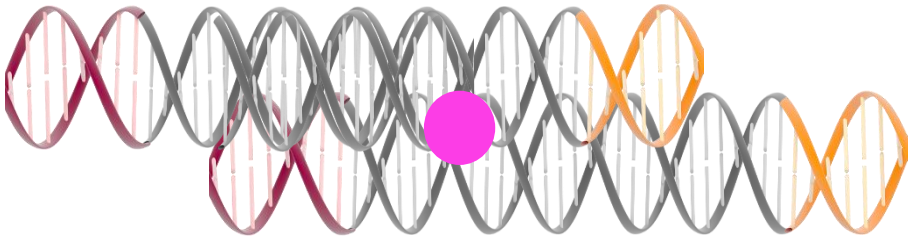
MDG gene panels for solid and hemato-oncological tumors

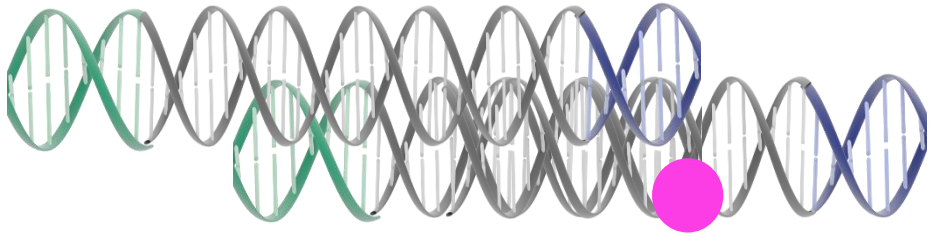
solid tumor panel (69 genes): *AKT1, ALK, APC, AR, BAP1, BRAF, BRCA1, BRCA2, CCND1, CDK4, CDK6, CDKN2A, CDKN2B, CTNNB1, DDR2, DICER1, DPYD, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FOXL2, FRK, GATA3, GNA11, GNAQ, GNAS, H3F3A, H3F3B, HIST1HB3, HIST1H3C, HNF1A, HRAS, IDH1, IDH2, IL6ST, JAK1, JAK2, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PIK3R1, POLE, PTEN, RB1, RET, RNF43, ROS1, SMAD4, SMARCA4, SMARCB1, SMO, SPOP, STAT3, STK11, TERT, TP53, VHL*

hemato-onco tumor panel (64 genes): *ANKRD26, ASXL1, ATM, BCL2, BCOR, BCORL1, BIRC3, BRAF, BTK, CALR, CBL, CEBPA, CRLF2, CSF3R, CUX1, DDX41, DNMT3A, EGR2, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA2, HRAS, IDH1, IDH2, IKZF1, IL7R, JAK2, JAK3, KIT, KRAS, MPL, NF1, NFKBIE, NOTCH1, NPM1, NRAS, PAX5, PHF6, PLCG2, POT1, PPM1D, PTPN11, RAD21, RPS15, RRAS, RUNX1, SETPB1, SF1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT5B, TET2, TP53, U2AF1, WT1, XPO1, ZRSR2*

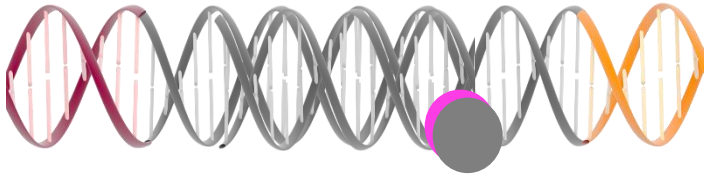
SeqCap library prep – capture-based NGS

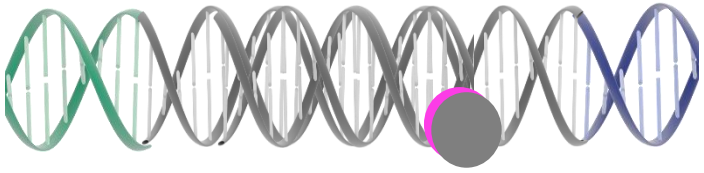
enrichment of DNA fragments with genes of interest





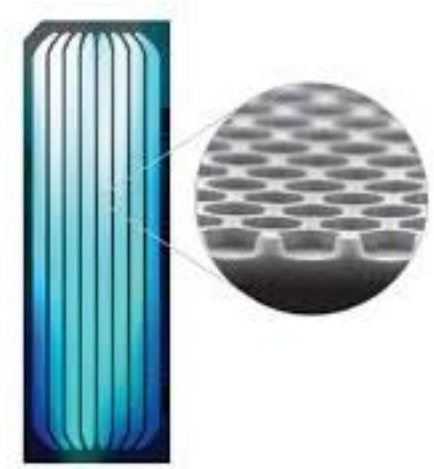
4) purification of biotinylated-probe bound DNA fragments with streptavidin-coated beads + amplification

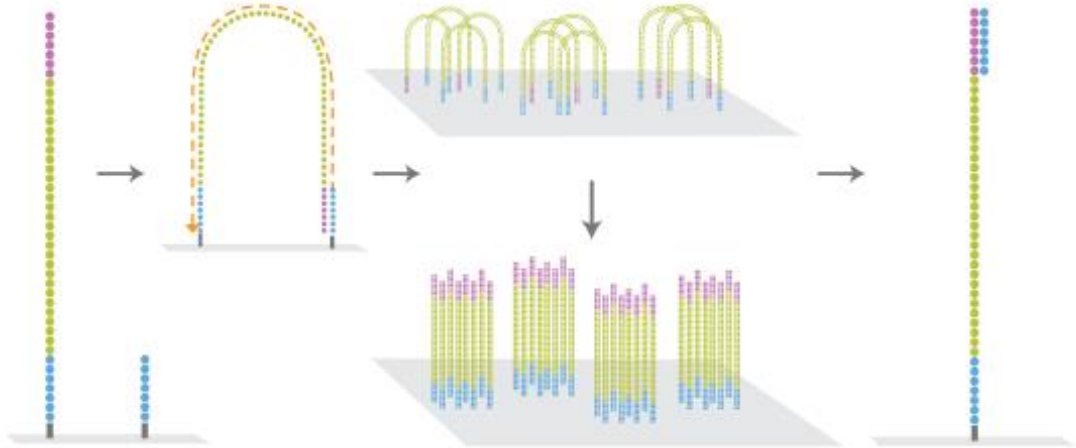


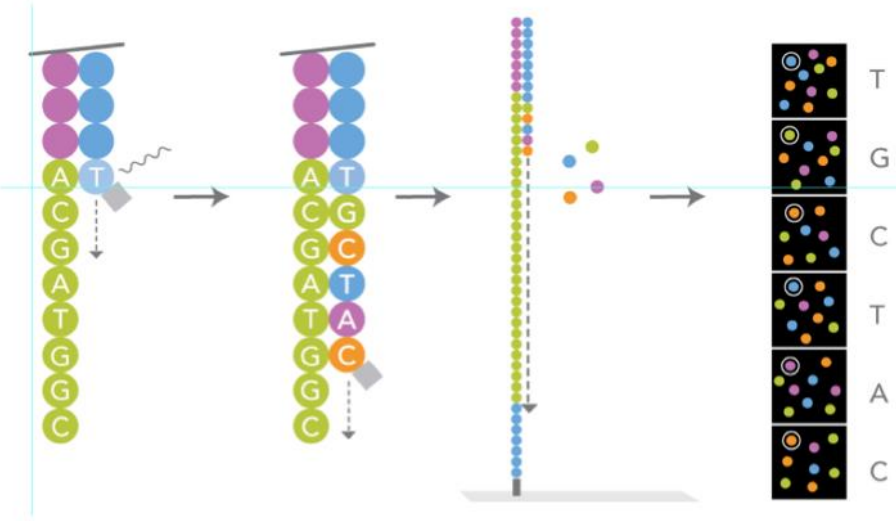


Illumina sequencing of SeqCap library prep

hybridisation, bridge amplification, cluster generation followed by sequencing-by-synthesis







Illumina sequencing van SeqCap library prep

sequencing instruments @CMGG

MiSeq
Illumina
up to 15 Gb

targeted sequencing

NextSeq 500
Illumina
up to 120 Gb

small/polyA/total RNA sequencing

HiSeq 3000
Illumina
up to 750 Gb

shallow whole genome/NIPT

NovaSeq
Illumina
up to 3000 Gb

exome/whole genome sequencing

NGS data-analysis

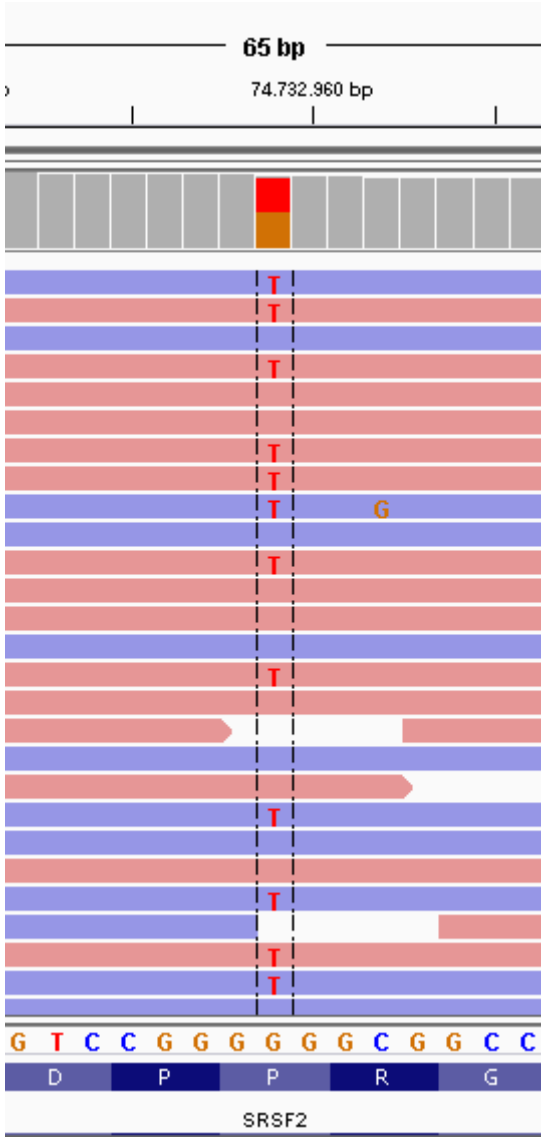
in-house bcbio datamining workflow

coverage:

- sequencing depth = amount of unique reads for a specific nucleotide in the sequencing data

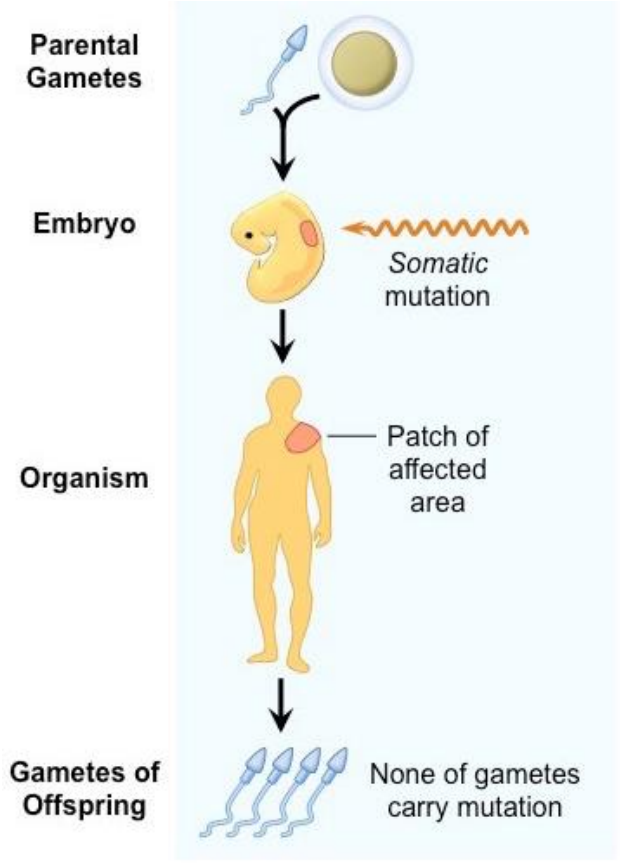
variant calling and reporting:

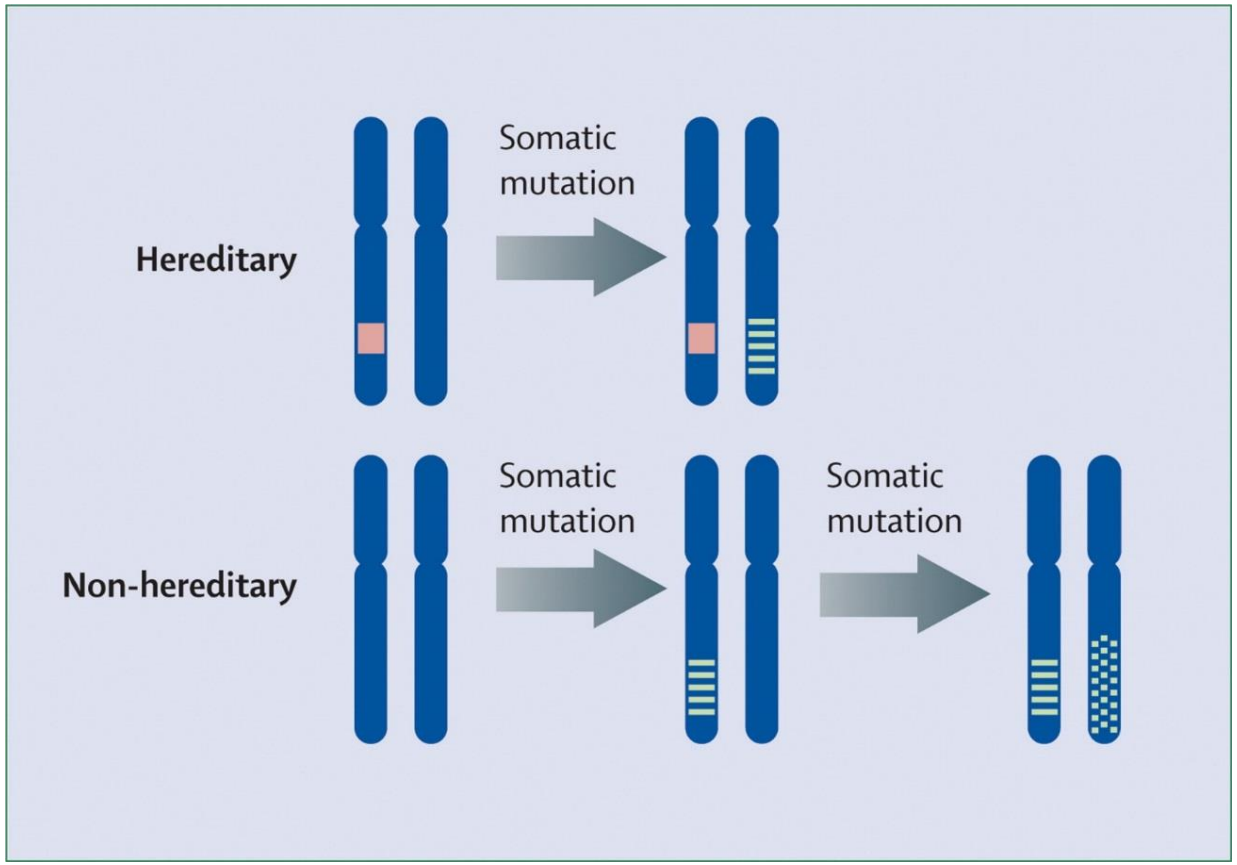
- ≥ 300X coverage & ≥ 5% VAF
- exception: 2-5% VAF known hotspot variants with variant present in >10 reads



SRSF2 c.284C>A (p.(Pro95His))
49% VAF

detection of somatic variants with NGS





Sharma et al. Nat Rev Cancer 2007

detection of somatic variants with NGS

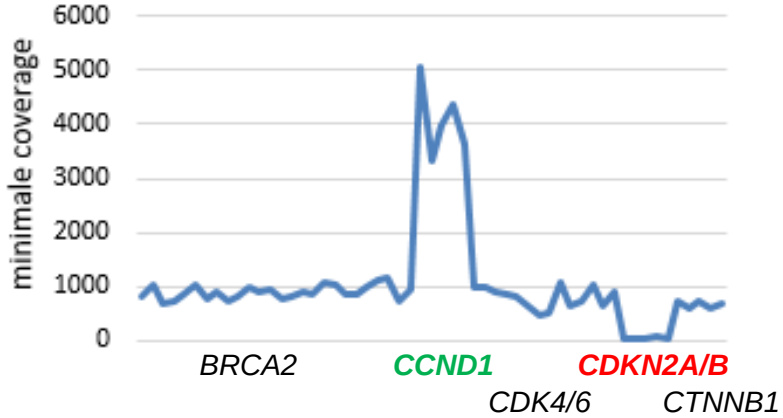
subsitutions (SNVs), deletions, insertions, copy number variants (CNVs) based on coverage

substitutions (SNV)

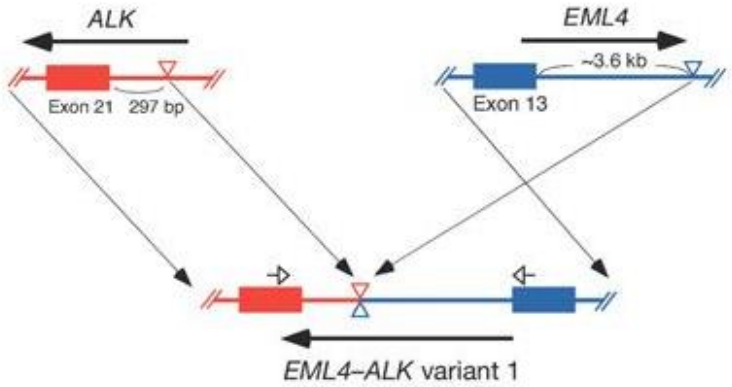
deletions

insertions

copy number variants (CNVs) based on coverage



fusions

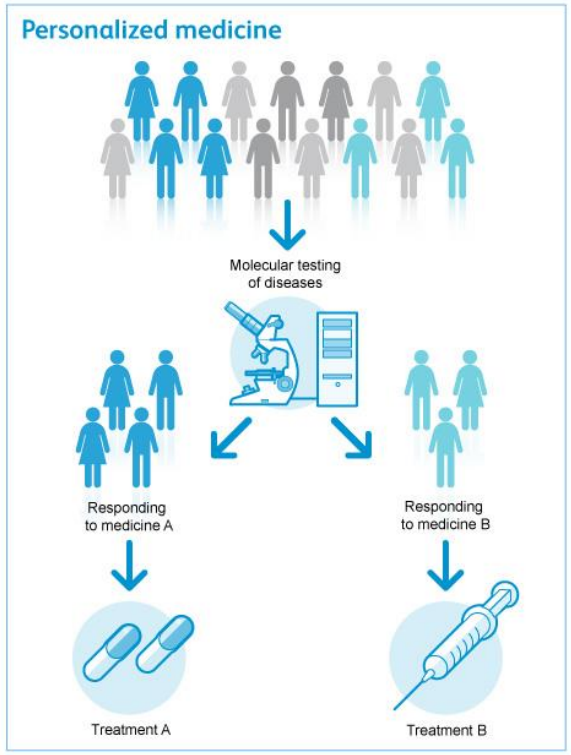


detection of somatic variants with NGS

prediction of therapy response to molecular drugs and immunotherapy
example. high-grade serous ovarian cancer *BRCA1-BRCA2* variants, melanoma *BRAF* variants, ER+ breast cancer *PIK3CA* variants

diagnosis
example. pancreatic cysts: *GNAS, KRAS, RNF43, VHL, CTNNB1* variants

prognosis
example. endometrial tumor: *POLE* variants, glioma: *TERT* promoter variants



New molecular and diagnostic technologies can be used to match select groups of patients with treatments that may give them the best results

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

MolecularDiagnostics.be + Scienscano: guidelines for harmonisation of variant classification/annotation/reporting

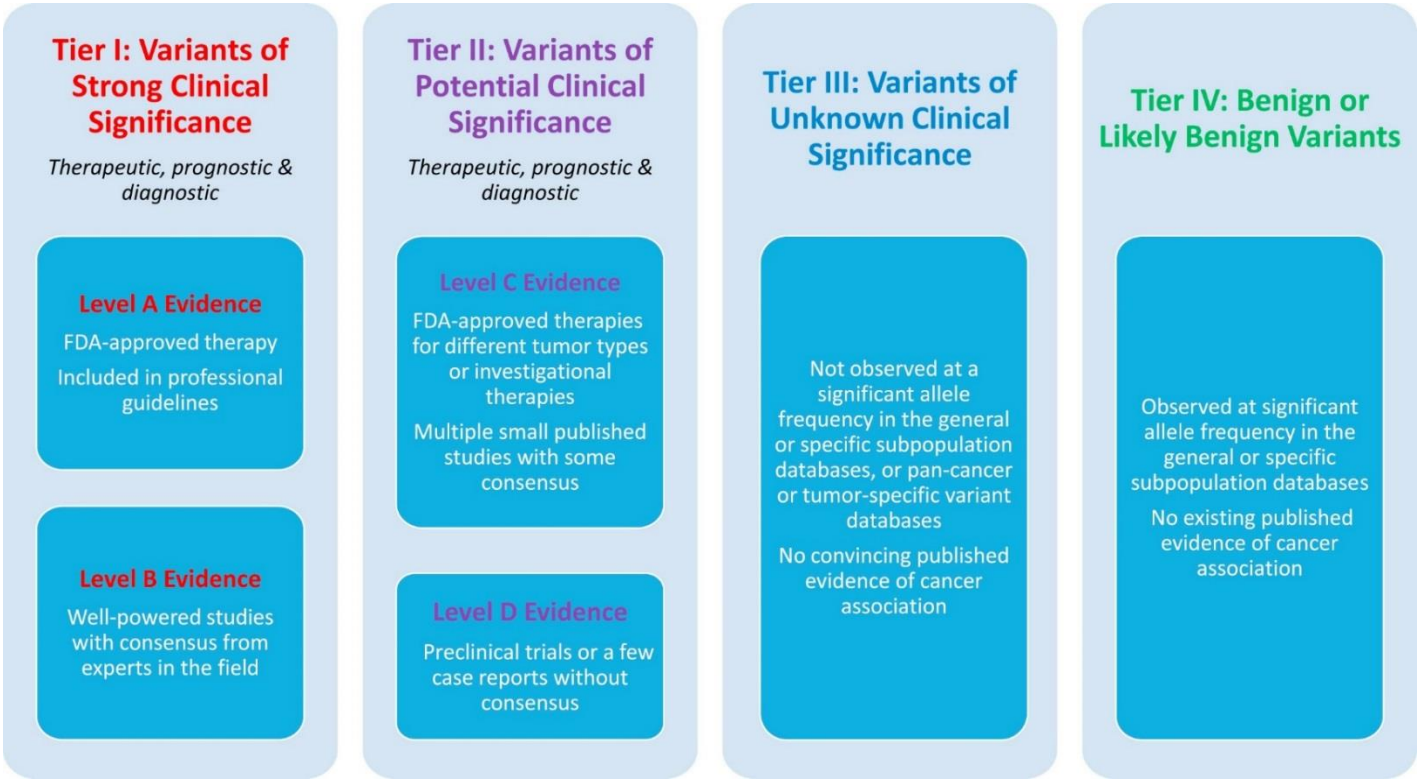
classification based on ACMG/AMP standards & guidelines (Richards et al. Genet Med 2015)

pathogenic	example. *BRAF* c.1799T>A p.(Val600Glu)
likely pathogenic	example. *PIK3CA* c.1357G>C p.(Glu453Gln)
VUS	example. *ALK* c.3513C>G p.(Ile1171Met)
likely benign	example. *ALK* c.4796C>A p.(Pro1599His)
benign	example. *TP53* c.215C>G p.(Pro72Arg)

reporting of pathogenic, likely pathogenic and VUS variants

variant interpretation of NGS data

clinical classification



→ recommended: reporting of variants tiers I – III, NOT tier IV

Li et al. J Mol Diagn 2017

NGS report

solid tumors

- pathogenic, probably pathogenic variants and variants of unknown significance (VUS) detected in **all 69 genes** are reported in **all solid tumor types**
  - *BRCA2* pathogenic variant in melanoma: precision2 clinical trials with olaparib @UZGent, germline mutation analysis recommended @CMGG
  - *DPYD* pathogenic variant in a colorectal tumor: germline mutation analysis recommended @CMGG, toxicity for 5-FU & capecitabine chemotherapy
  - *FGFR2* pathogenic variant in an endometrial tumor: FGFRi clinical basket trials @UZGent

reimbursement in Belgium – NGS convention

NGS convention – extra fee for NGS tests in selection of solid tumors (350 euro) <> 210 euro

tumor types in NGS convention

→ obligation to test minimal gene list per tumor type with NGS

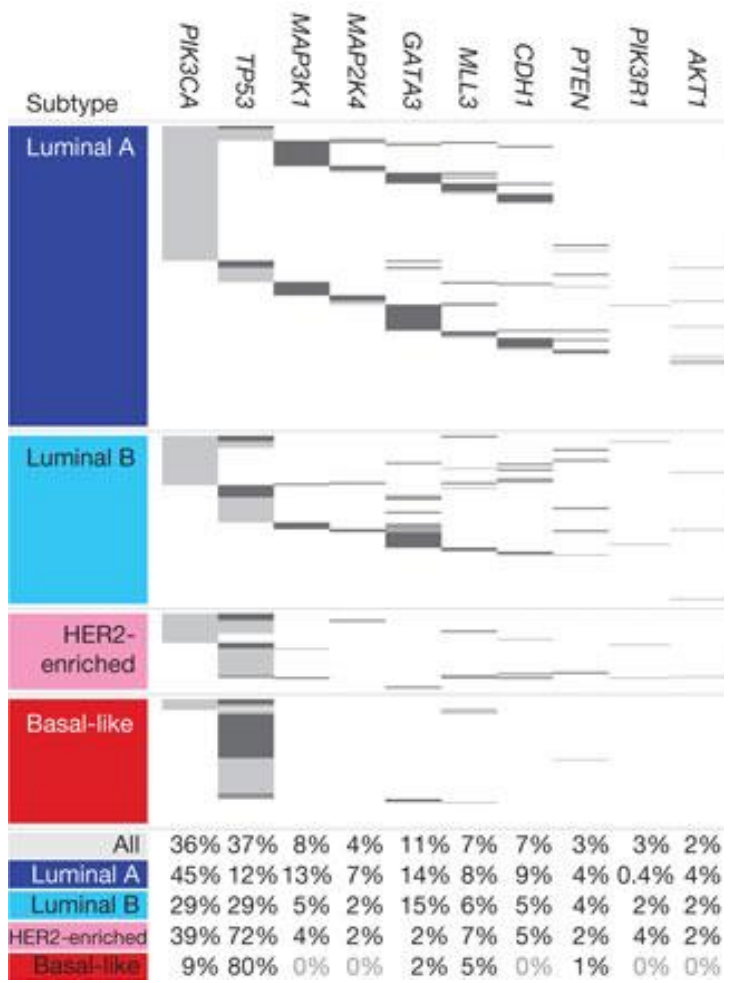
- high-grade non-mucinous epithelial ovarian cancer
- melanoma stage III metastatic lymph nodes / metastatic
- glioma (IDH1- on IHC)
- medulloblastoma
- thyroid carcinoma (bethesda class 3 or 4)
- metastatic colorectal carcinoma
- pancreatic carcinoma - cysts
- GIST
- lung carcinoma (non-squamous carcinoma or squamous carcinoma non/seldom-smoker or progressive under targeted therapy)

casus 1

patient with metastatic ER+ HER2- breast cancer, 57 years old – tumour sample with 40% TC

- *PIK3CA* c.3140A>G p.(His1047Arg) 24% VAF: pathogenic variant
- *TP53* c.833C>T p.(Pro278Leu) 41% VAF: pathogenic variant
- *BAP1* c.1039C>T p.(His347Tyr) 50% VAF: VUS

- compassionate use programma / clinical study: alpelisib (PIK3CA inhibitor) in combination with fulvestrant/letrozole for *PIK3CA* mutated ER+ HER2- breast cancer patients



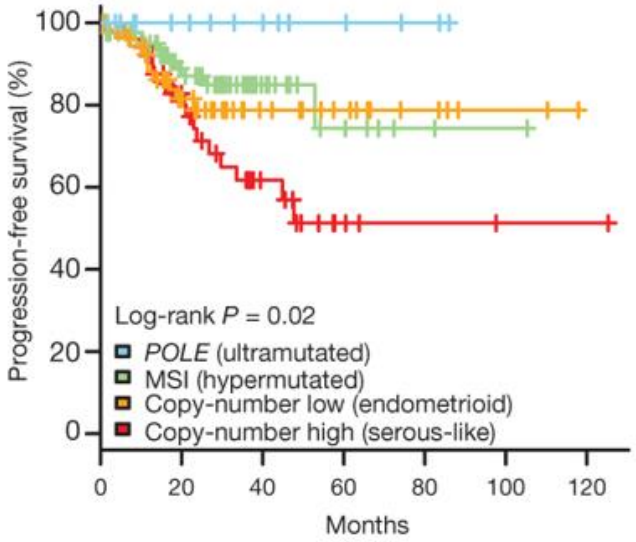
Koboldt et al. Nature 2012

casus 2

patient with endometrioid endometrial cancer, 51 years old – tumour sample with 80% TC

- *POLE* c.857C>G p.(Pro286Arg) 40% VAF: pathogenic variant
- *BRCA2* c.7795G>T p.(Glu2599Ter) 42% VAF: pathogenic variant
- *CTNNB1* c.104T>G p.(Ile35Ser) 45% VAF: pathogenic variant
- *PTEN* c.19G>T p.(Glu7Ter) 41% VAF: likely pathogenic variant
- *PTEN* c.895G>T p.(Glu299Ter) 40% VAF: likely pathogenic variant
- *PIK3R1* c.1042C>T p.(Arg348Ter) 80% VAF: likely pathogenic variant
- *ESR1* c.1610A>C p.(Tyr537Ser) 41% VAF: pathogenic variant

  + 22 VUS variants
- *POLE* mutated endometrial cancer group are associated with good prognosis



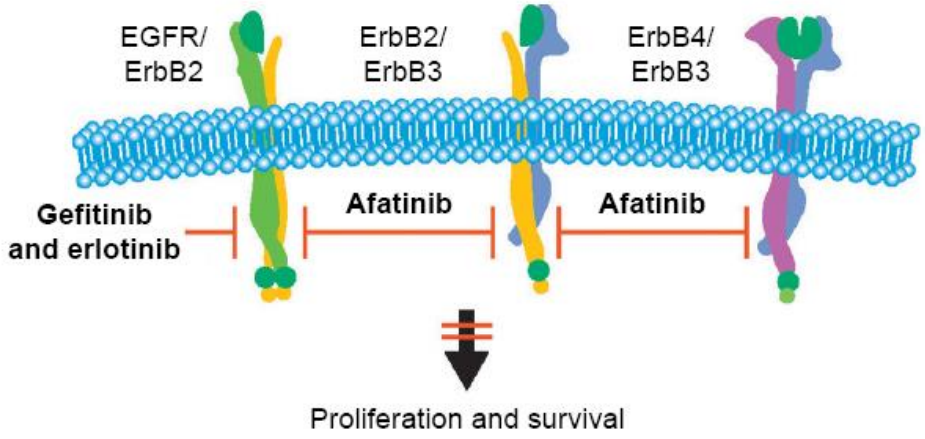
TCGA et al. Nature 2013

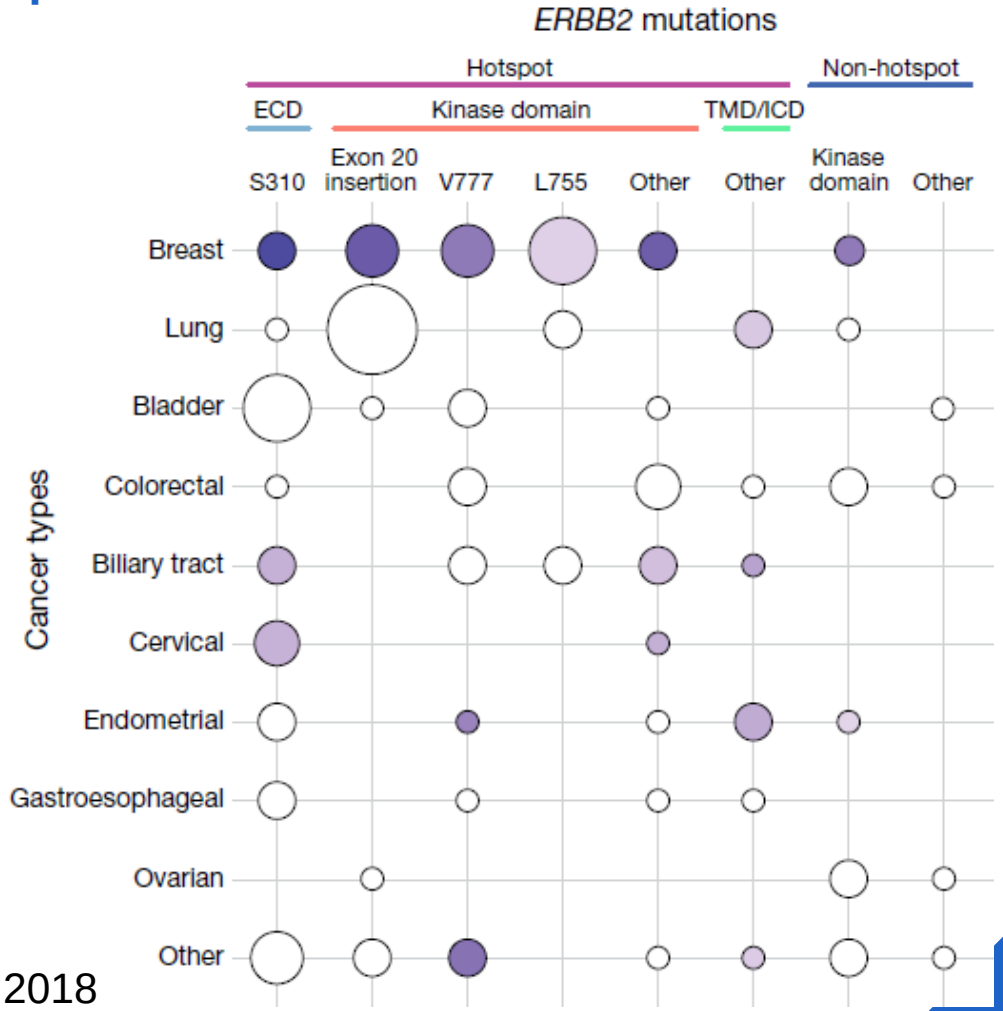
casus 3

patient with ovarian carcinoma, 43 years old – sample with 50% TC

- *ERBB2* c.2314_2325dup p.(Tyr772_Ala775dup)
  37% VAF: pathogenic variant

- PRECISION2 trial: afatinib





Hyman et al. Nature 2018

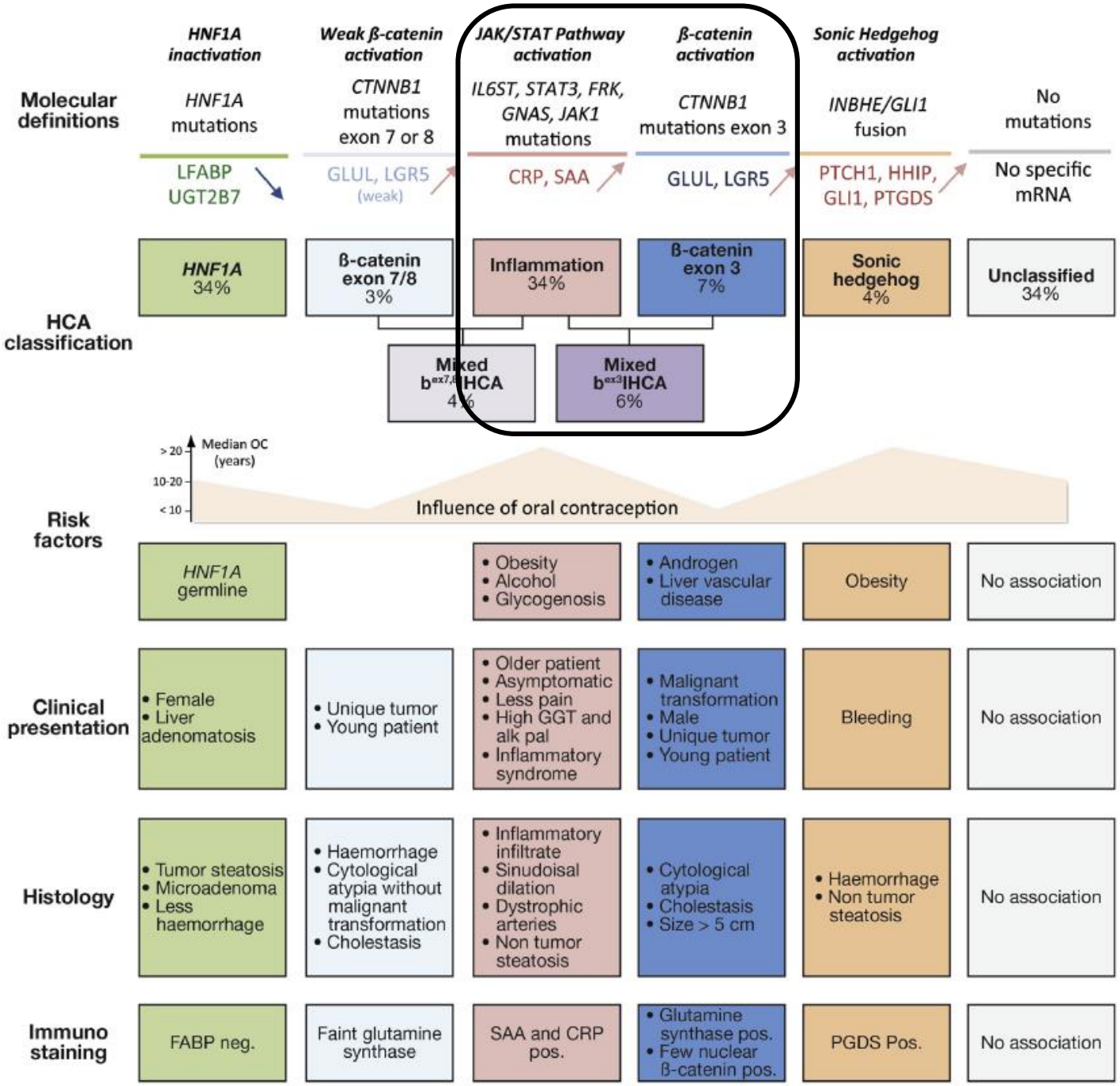
casus 4

patient with hepatocellular adenomas, 25 years old – sample with 80% TC

- *CTNNB1* c.133T>C (p.(Ser45Pro)) 34% VAF: pathogenic variant
- *IL6ST* c.567_578del (p.(Tyr190_Asn193del)) 21% VAF: pathogenic variant

classification hepatocellular adenoma

→diagnosis: mixed b^{ex3}IHCA



Nault et al. Gastroenterology 2017

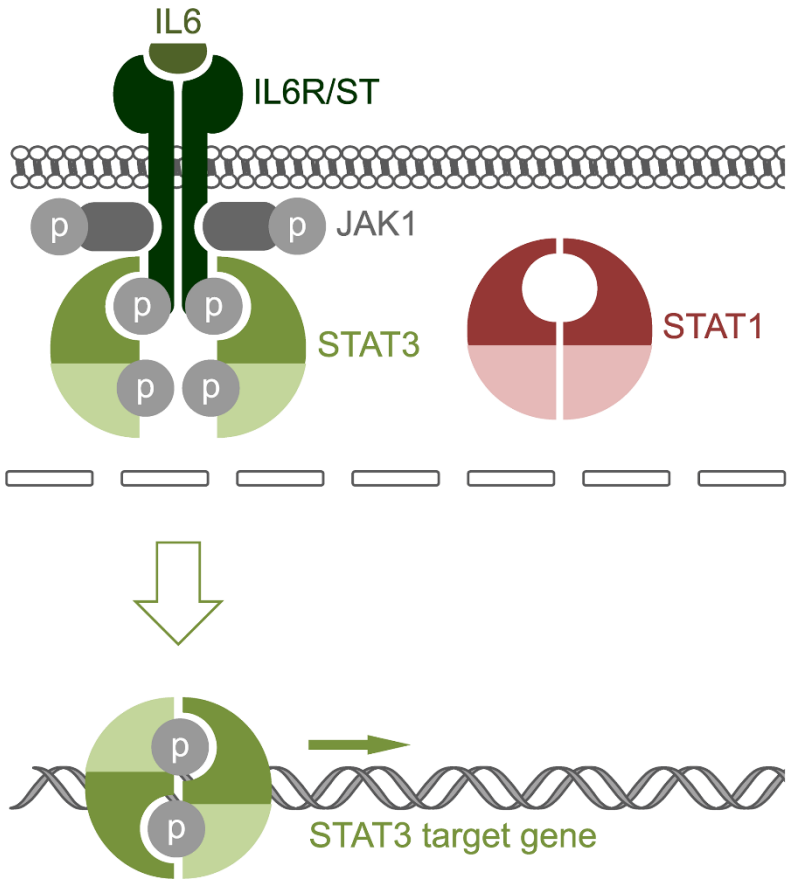
casus 4

patient with hepatocellular adenomas, 25 years old – sample with 80% TC

- *CTNNB1* c.133T>C (p.(Ser45Pro)) 34% VAF: pathogenic variant
- *IL6ST* c.567_578del (p.(Tyr190_Asn193del)) 21% VAF: pathogenic variant

→ diagnosis: mixed b^{ex3}IHCA

→ ruxolutinib (JAK1/JAK2 inhibitor): can be efficient in supressing IL6/JAK/STAT pathway activation due to *IL6ST* mutations (Nault et al. Gastroenterology 2017)



JONI VAN DER MEULEN
supervisor MDG, molecular biologist
Molecular Diagnostics UZ Gent (MDG)
+32 (0)9 332 39 71

Universitair Ziekenhuis Gent
C. Heymanslaan 10 | B 9000 Gent
T +32 (0)9 332 21 11
E info@uzgent.be

www.uzgent.be
Volg ons op

UZ GENT
UNIVERSITEIT GENT