# *Homologous recombination repair deficiency*

# HRD


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## Andreas Jung conflict of interest:

Honoraria for

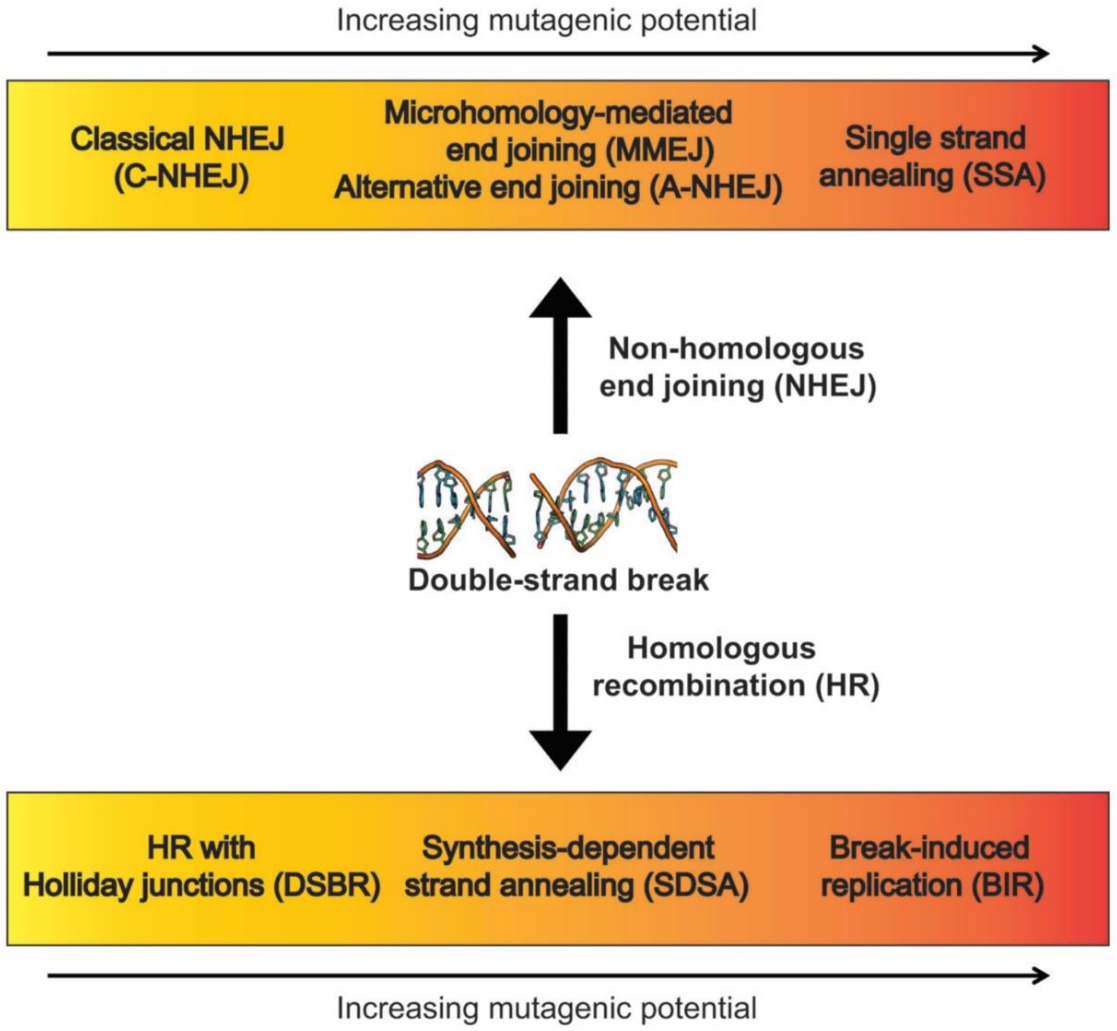
- advisory boards
- Talks
- travel reimbursement

Amgen, AstraZeneca, Bayer Pharmaceuticals, BMS, Biocartis, Boehringer Ingelheim, Merck KgA, Lilly, MSD, Novartis, QuIP GmbH, Roche Pharma, Takeda, Thermo Fisher

# Outlook

- The WWW of HRD
  - **W**hat is it?
  - **W**hy is it important?
  - **W**hat is the benefit of knowing HRD?
- Signatures: HRD… - Next Generation Biomarkers
- How much is enough? Cut off for defining defects in HRR
- **O**ncology researchers **C**are **A**bout a **plus** of genetic information for comprehensive insights

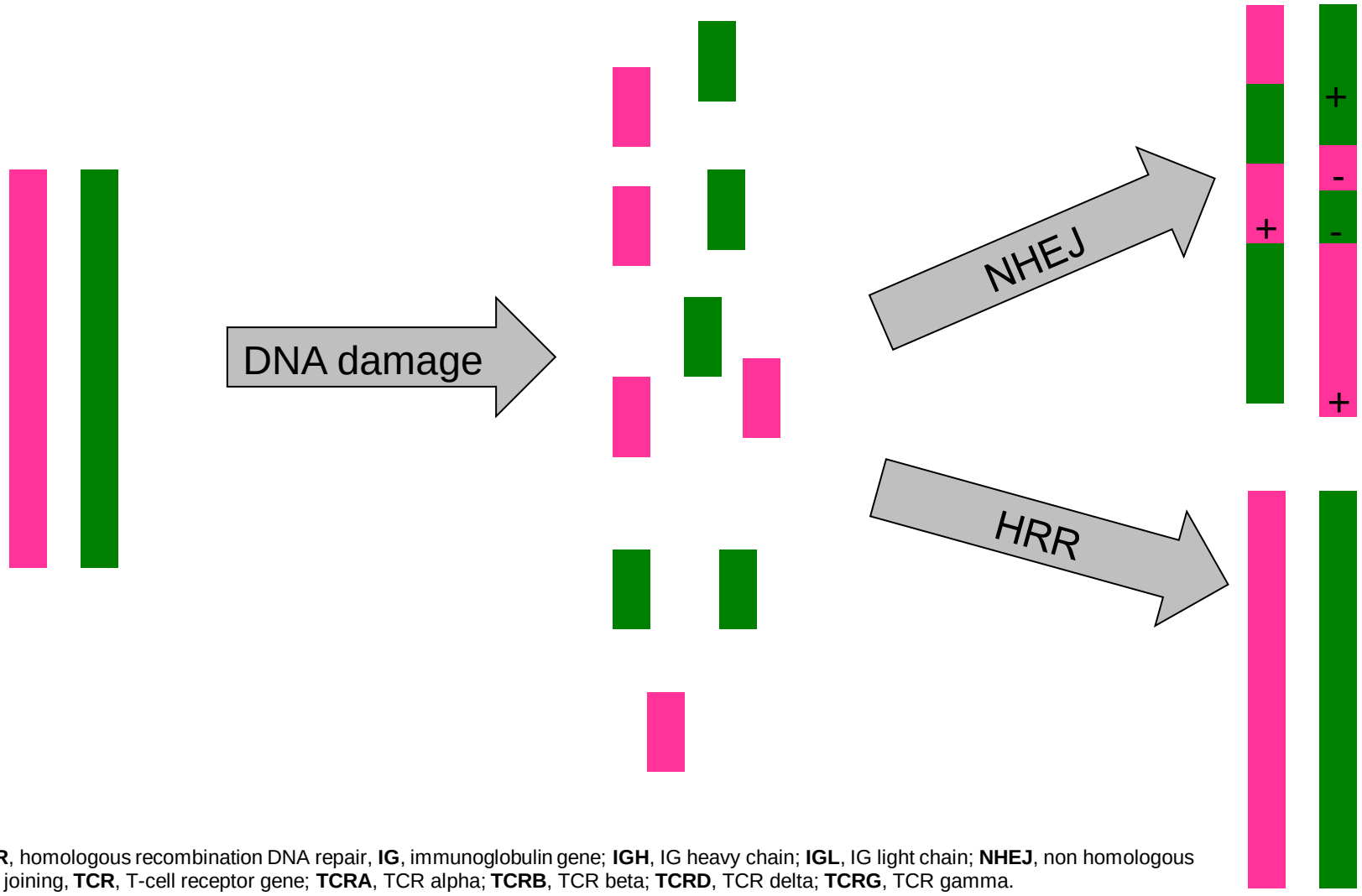
# DNA Double Strand Repair




**DSBR**, double strand repair.

Rodgers J Cell Physiol 231 15 (2016)

# Homologous recombination DNA-repair (HRR)




**HRR**, homologous recombination DNA repair, **IG**, immunoglobulin gene; **IGH**, IG heavy chain; **IGL**, IG light chain; **NHEJ**, non homologous end joining, **TCR**, T-cell receptor gene; **TCRA**, TCR alpha; **TCRB**, TCR beta; **TCRD**, TCR delta; **TCRG**, TCR gamma.

**NHEJ** is a more error prone process than HRR

- Used in V(D)J-joining
  - IGH
  - IGL
  - TRCRA
  - TCRB
  - TCRG
  - TCRD

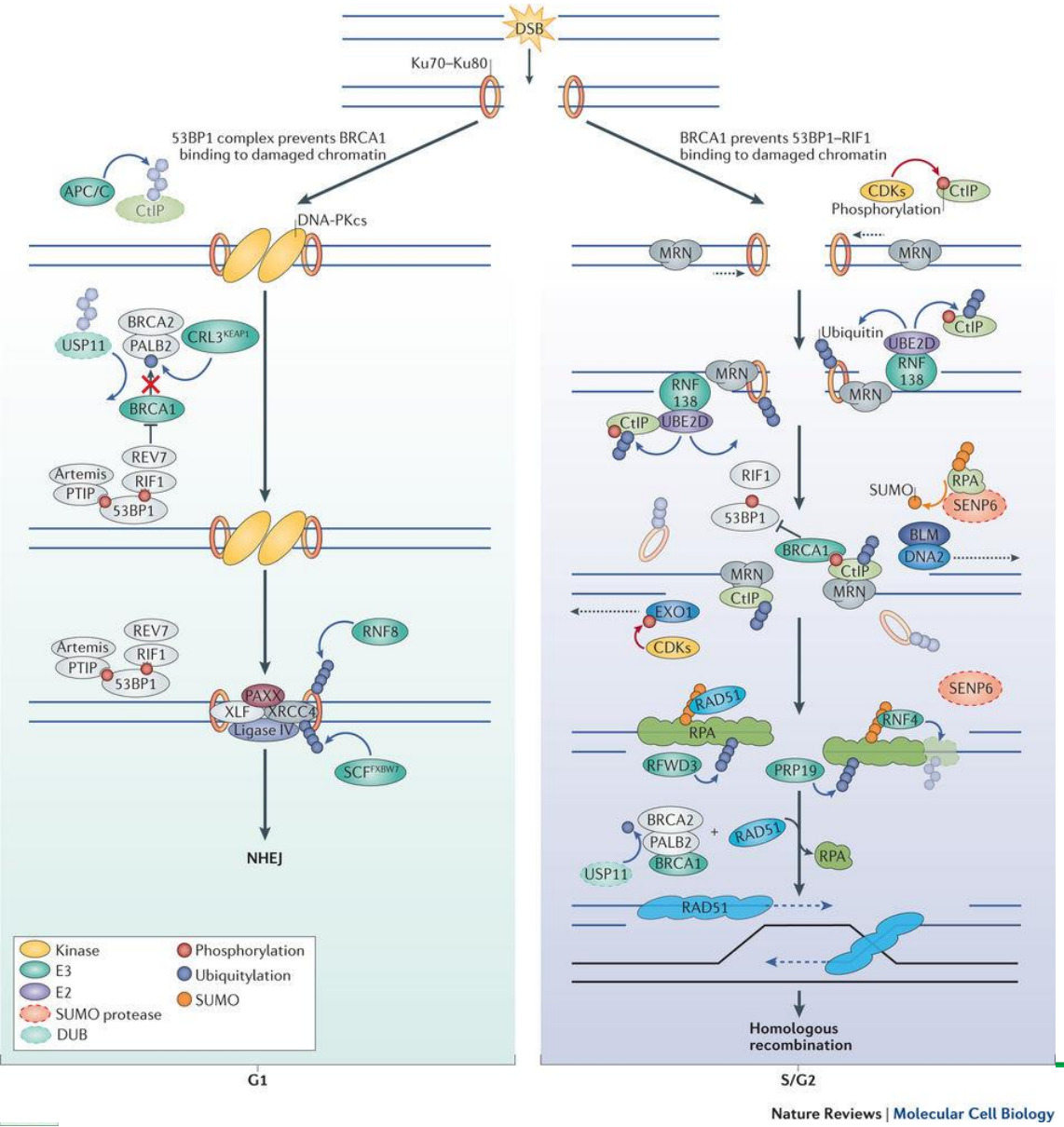
⇒ gains and losses of DNA

**HRR** is an exact(er) working mechanism

- Used in meiotic crossing over
- Complex not completely understood mechanism

Rodgers J Cell Physiol 231 15 (2016), A. Jung

# Homologous Recombination DNA-Repair




- Complex repair
- 41 genes
- Many tumor suppressor genes

→ Syndromes

- BRCA1, BRCA2

**HRR** is quite a complex process

- Functional role
- Interaction
- Competeness

of participating proteins has not been fully elucidated

***Mutations*** in components of HRR

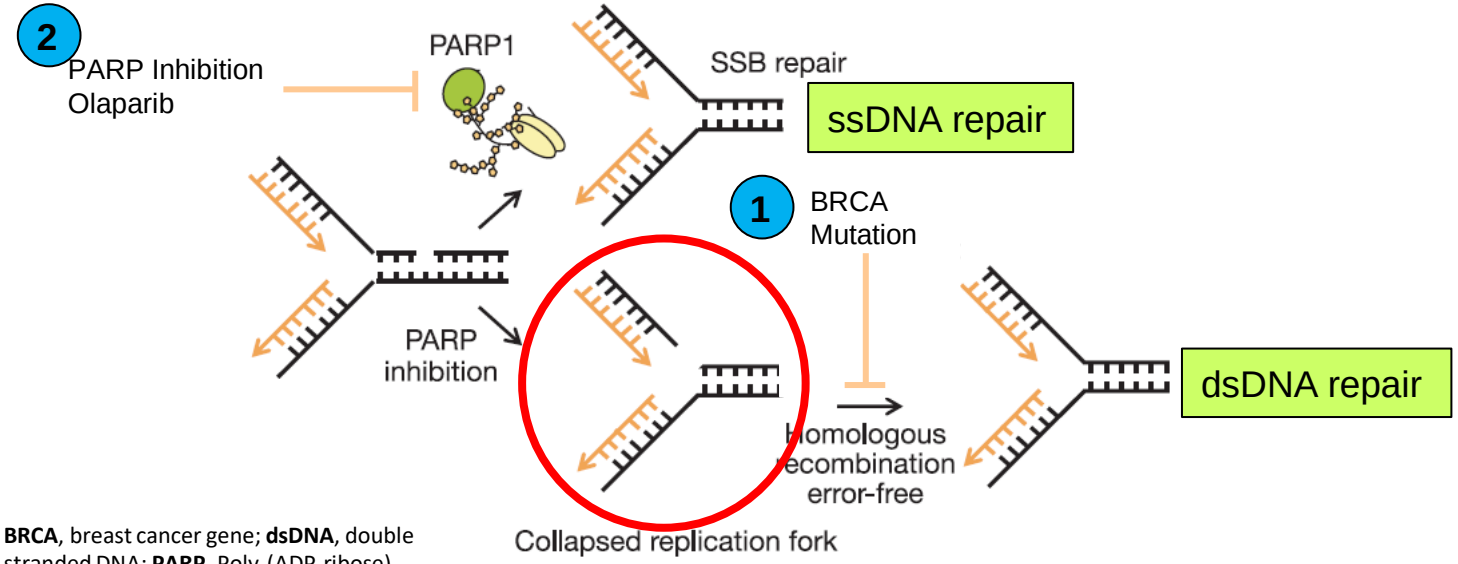
- Variant interpretation is difficult

→ Mostly stemming from germline diseases

Schwertmann Nat Rev Cell Biol 2016

# PARP inhibitors and HRD related tumor samples

| Substance | Study | Tumor Type | Biomarker |
|---|---|---|---|
| olaparib | PAOLA1 (NCT02477644) | ovarian cancer, … | BRCA1, BRCA2, **HRD** (1) |
| olaparib | PROfound (NCT02987543) | prostate | BRCA1, BRCA2, **HRD** (2) |




**BRCA**, breast cancer gene; **dsDNA**, double stranded DNA; **PARP**, Poly-(ADP-ribose) polymerase; **SSB**, single strand break; **ssDNA**, single stranded DNA.

- BRCA$^{-/-}$ cell lines are hypersensitive against PARP inhibitors[1,2]
- Modell
  (1) loss of *ds*DNA break repair (HRR) by mutation of e.g. BRCA1/ BRCA2
  (2) PARP is essential for *ss*DNA repair

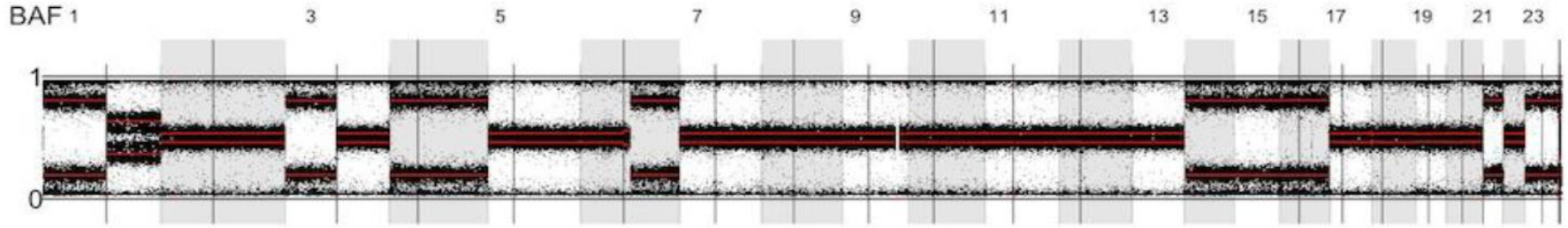
  → collapse of the replication fork results in cell death

(1) Ray-Coquard New Engl J Med 381 2416 (2019), (2) deBono New Engl J Med 382 2091 (2020), Hussain New Engl J Med 383 2345 (2020), Bryant Nature 434, 913 (2005), Farmer Nature 434, 917 (2005)

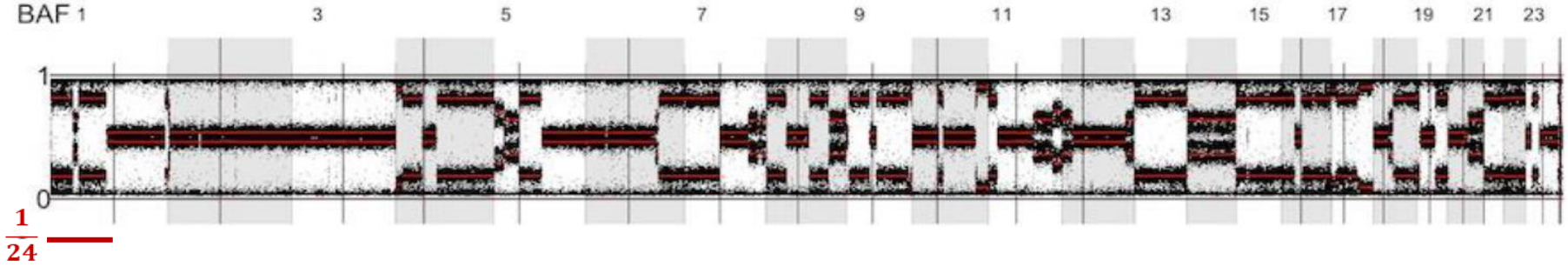
# Chromosomal rearrangemet in HGS Ovarian Cancer with HRD

Illumina: Infimum Cyto Array (*Pathologie – TUM; Prof. Dr. Wilko Weichert*)


## LGS HR intact



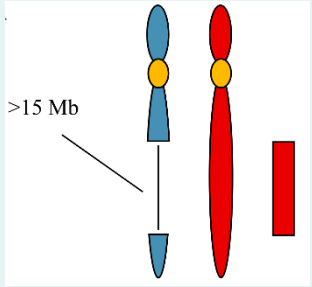
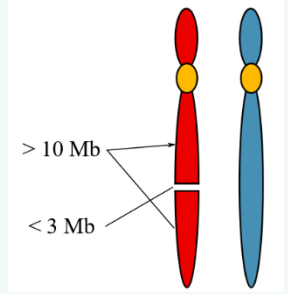
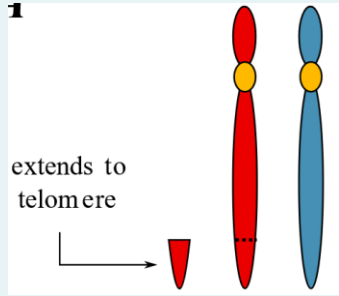

## HGS HR deficient BRCA2mut




**BAF**, B allele frequency.

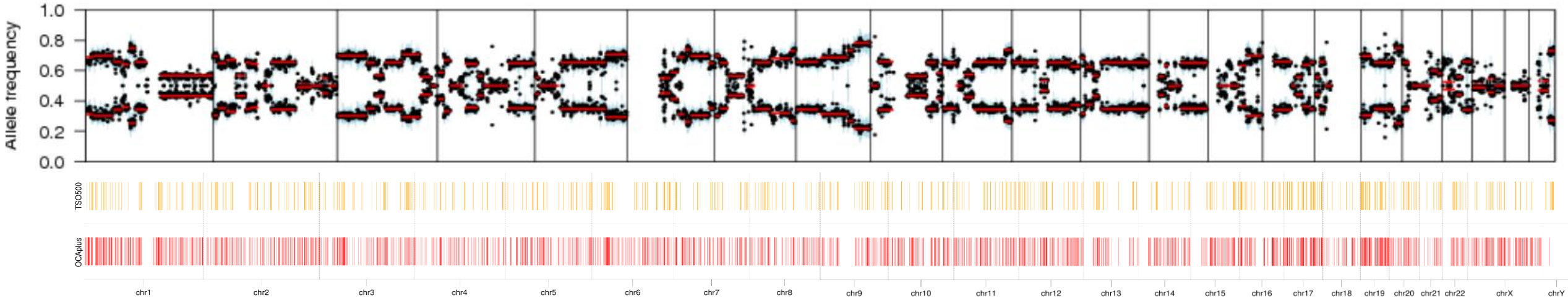
Illuimna; Mapagu, Westmead Institute, Sydney, Australia

# Some methods for determination of HRD

| | Type of measurement | Definition | Graphical |
|---|---|---|---|
| **LOH** | **Loss of heterozygosity** | number of 15 Mb exceeding LOH regions which do not cover the whole chromosome |   |
| **LST** | **Large scale transition** | chromosomal break between adjacent regions of at least 10 Mb, with a distance between them not larger than 3Mb |   |
| **TAI** | **Telomere allelic imbalance** | number AIs that extend to the telomeric end of a chromosome. |   |

Sztupinski Breast Cancer 4 16 (2018), Watkins Breast Cancer Res 16 211 (2014)

# Chromosomal rearrangemet in HRD can be detectet by coarse coverage of the genome




- OCA plus broader coverage of the genome

⇒ sufficient for detection of breakpoints (*NHEJ is a coarse granular genetic rearrangement*)
⇒ HRD cut off values of different detection methods are comparable

Coverages of genome: **Bioconductor** (https://github.com/Bioconductor; https://bioconductor.org/packages/release/bioc/), **RtrackLayer** (https://www.bioconductor.org/packages//2.10/bioc/html/rtracklayer.html)

Mapagu, Westmead Institute, Sydney, Australia; Albig Institute for Pathology, TUM, Munich, Germany (courtesy Nicole Pfarr)

# PARP inhibitors and HRD related tumor samples

| Substance | Study | Tumor Type | Biomarker |
|---|---|---|---|
| olaparib | PAOLA1 (NCT02477644) | ovarian cancer, … | BRCA1, BRCA2, **HRD** (1) |
| olaparib | PROfound (NCT02987543) | prostate | BRCA1, BRCA2, **HRD** (2) |

| Vendor | Content of test | Cut off |
|---|---|---|
| M | **BRCA1, BRCA2, LOH, LST, TAI** | **42** |
| F | 324 genes, TMB, MSI, **LOH** | |

**Consortia: France, Germany, …**

Measuring cut offs for several tests: … OCAplus, …Myriad MyChoice

(1) Ray-Coquard New Engl J Med 381 2416 (2019), (2) deBono New Engl J Med 382 2091 (2020), Hussain New Engl J Med 383 2345 (2020)

# OCA plus

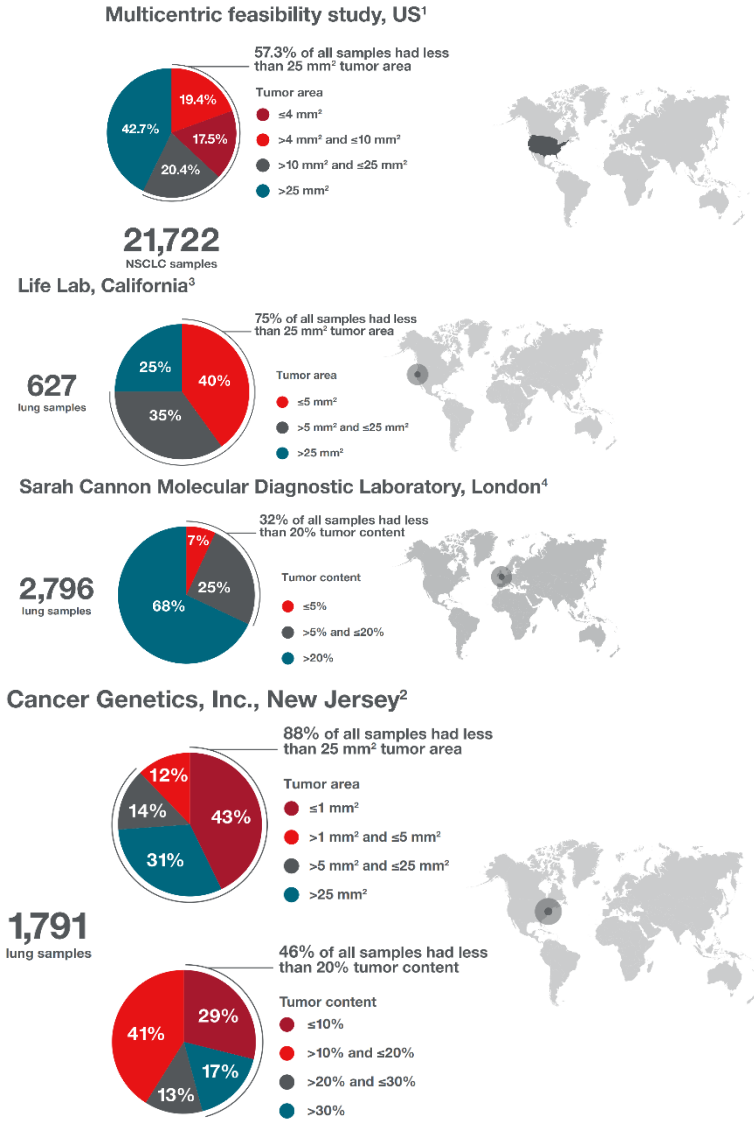
**Oncomine Comprehensive Assay plus for CGP and HRD research**

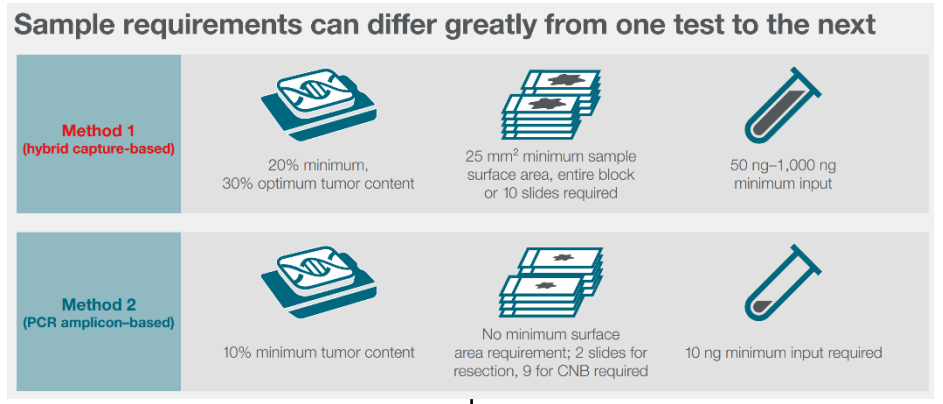
**O**ncology researchers **C**are **A**bout a **plus** of genetic information for comprehensive insights

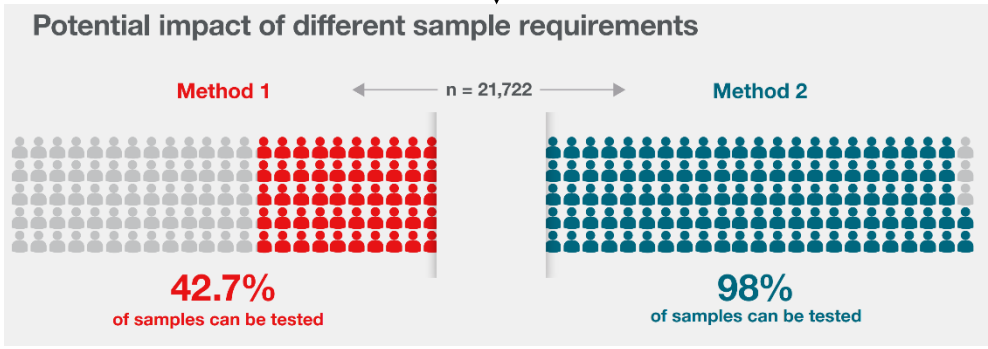
| Goal | Oncomine Comprehensive Plus | TruSight Oncology 500 |
|---|---|---|
| Biomarker DNA-mutations (SNVs, InDels, CNVs) | 500 Genes | 524 Genes |
| RNA-gene fusion | 👍 *Has to be added (free choice!)* | 👍 *Comes in bundle: TST170* |
| TMB | 👍 | 👍 |
| MSI | 👍 | 👍 |
| HRD | 👍 | 👎 |
| Amount of Nucleic Acid | 👍 | 👎 |
| Analysis pipeline (1°, 2°, 3° analysis) | 👍 *Ion Reporter* *Oncomine Reporter (OR)* | 👎 |

For research use only. Not for use in diagnostic procedures

# Advantage of amplicon based NGS detection




Scott, ASCO (2020), *CAP TODAY*, Moore *Pathol* 2018), Thermo Fisher Flyer (COL012749 0820)

# Primary Analysis

| S1-0469-25-5165 | fnd | | type | | S | I | E | R | Phred | VF |
|---|---|---|---|---|---|---|---|---|---|---|
| ARID1A-Gen: chr1:27100181, Exon 16, c.3999_4001delGCA (NM_006015.6)-Ref:, p.Gln1334del, Abdeckung (coverage): 1883, Allel Frequenz (MUT): 4.09%, ClinVar:- | 1 | OFF | TuSu | | | | | | 142 | 4.1 |
| OR2T33-Gen: chr1:248436616, Exon 1, c.500_501delGTinsAC (NM_001004695.1)-Ref:, p.Cys167Tyr, Abdeckung (coverage): 299, Allel Frequenz (MUT):%, ClinVar:- | 1 | | | | | | | | 5278 | |
| SDHA-Gen: chr5:228363, Exon 6, c.685G>A (NM_004168.4)-Ref:, p.Gly229Arg, Abdeckung (coverage): 78, Allel Frequenz (MUT): 7.69%, ClinVar:3 | 1 | | TuSu | | | | | | 33 | 7.7 |
| HLA-A-Gen: chr6:29911255, Exon 3, c.555delT (NM_001242758.1)-Ref:, p.Asp185GlufsTer29, Abdeckung (coverage): 34, Allel Frequenz (MUT): 35.29%, ClinVar:- | 1 | | | | | | | | 113 | 35.3 |
| CDK6-Gen: chr7:92404036, Exon 3, c.343C>G (NM_001145306.2)-Ref:, p.Pro115Ala, Abdeckung (coverage): 1999, Allel Frequenz (MUT): 45.72%, ClinVar:- | 1 | | TuSu | | | | | | 10778 | 45.7 |
| MUC12-Gen: chr7:100635418, Exon 2, c.1574A>G (NM_001164462.1)-Ref:, p.His525Arg, Abdeckung (coverage): 24, Allel Frequenz (MUT): 37.5%, ClinVar:- | 1 | | | | | | | | 71 | 37.5 |
| RB1-Gen: chr13:48955390, Exon 17, c.1507_1528delTCTCAGAATCTTGATTCTGGAA (NM_000321.2)-Ref:, p.Ser503GlnfsTer9, Abdeckung (coverage): 205, Allel Frequenz (MUT): 16.59%, ClinVar:- | 1 | | TuSu | | | | | X | 2569 | 16.6 |
| ZFHX3-Gen: chr16:72821362, Exon 10, c.10813C>T (NM_006885.4)-Ref:, p.Pro3605Ser, Abdeckung (coverage): 21, Allel Frequenz (MUT): 28.57%, ClinVar:- | 1 | | TuSu | | | | | | 38 | 28.6 |
| TP53-Gen: chr17:7577534, Exon 7, c.747G>C (NM_000546.5)-Ref:, p.Arg249Ser, Abdeckung (coverage): 1983, Allel Frequenz (MUT): 71.34%, ClinVar:3 | 1 | | TuSu | | | | | | 17077 | 71.3 |
| MAP2K4-Gen: 17p12, chr17:11924164, etwa 18.45-fache Vermehrung | | | TuSu | | | | | X | | |
| NCOR1-Gen: 17p12p11.2, chr17:15935586, etwa 11.7-fache Vermehrung | | | | | | | | X | | |
| ZRSR2-Gen: chrX:15841227, Exon 11, c.1313_1314delGCinsCAGCCGG (NM_005089.3)-Ref:, p.Gly438AlafsTer?, Abdeckung (coverage): 1165, Allel Frequenz (MUT): 75.49%, ClinVar:- | 1 | | | | | | | | 10154 | 75.5 |

- technology (chemistry) produces error, which have to be wiped out

  *Ion Torrent: prone for A/Ts, Illumina prone for C/Gs*

→ inspection of primary calls is essential

→ results in index-lists showing artifacts

→ Mutations

- found in (almost) all cases
- found in reads of one but not another overlapping amplicon
- often found at the ends
- show strand bias
- Often have a low(er) allele frequency

| Exception-S | ExceptList-S | ExceptDecis-S |
|---|---|---|
| ABCB1 | c.-330-27755G>A | OFF |
| AGK-BRAF | | OFF |
| ALK | c.3598G>C | OFF |
| ARID1A | c.2524_2525insC | OFF |
| ARID1A | c.3361G>A | OFF |
| ARID1A | c.3999_4001delGC | OFF |
| ATM | c.161_162insC | OFF |
| ATM | c.2080_2122delCT | OFF |
| ATM | c.5948A>G | OFF |
| ATRX | c.1275_1276insA | OFF |
| ATRX | c.2785C>G | OFF |
| ATRX | c.4055_4056insA | OFF |

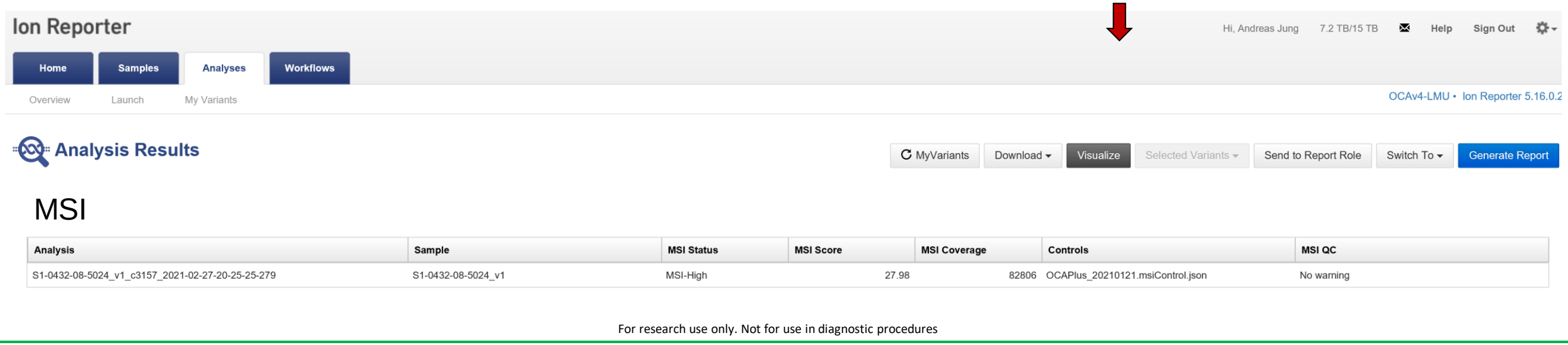
# Variant interpretation (HRR centered)

| Database/ resource | URL | Content |
|---|---|---|
| BRCA Exchange | https://brcaexchange.org/ | 5 class tier<br>1 –benign, 2-likely benign, 3-VUS, 4- likely pathogenic, 5-pathogenic |
| ClinVar | https://www.ncbi.nlm.nih.gov/clinvar/ | Curated database, refreshed weekly |
| FLOSSIES | https://whi.color.com/ | Fabulous ladies over seventies<br>→ Variations without pathogenic relevance |
| VEST @ Karchin lab | https://karchinlab.org/apps/appVest.html | Variant effect scoring tool (machine learning) |
| fathmm<br>Functional Analysis through Hidden Markov Models (v2.3) | http://fathmm.biocompute.org.uk/ | Score based on statistical Markov modelling |
| CADD - Combined Annotation Dependent Depletion<br>… | https://cadd.gs.washington.edu/ | Combined Annotation Dependent Depletion<br>- Combination of different sources and modells to annotate pathogenicity score |

Nono BMC Med Genomics 12 22 (2019)

# OCA Plus Results

| Case | MSI | TMB | LOH | MAPD | MSS | TMB | Gene | Chr | Position | Exon | cDNA | Protein | NM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5036 | 6.09 | 6.66 | **68.08** | 0.338 | MSS | low | BRCA2 (class 5) | 13 | 32913269 | 11 | c.4777G>T | p.E1593* | NM_000059.3 |
| 5040 | 3.01 | **13.24** | 20 | 0.293 | MSS | **high** | FANCA | 16 | 89807256 | 38 | c.3781_3783del | p.Phe1263del | NM_00135 |
| 5060 | 3.11 | 2.85 | **42.32** | 0.203 | MSS | low | BRCA1 (class 5) | 17 | 41246041 | 10 | c.1504_1507delTTAA | p.Leu502Sfs*21 | NM_007294.3 |
| 5041 | 2.97 | 3.81 | 4.7 | 0.296 | MSS | low | MSS | | | | | | |
| 5066 | 12.02 | 7.58 | **50.42** | 0.177 | MSS | low | BRCA1/2 WT | | | | | | |
| 5024 | **27.98** | 2.85 | 18.49 | 0.377 | **MSI** | low | MLH1 methylated | | | | | | |




## MSI

| Analysis | Sample | MSI Status | MSI Score | MSI Coverage | Controls | MSI QC |
|---|---|---|---|---|---|---|
| S1-0432-08-5024_v1_c3157_2021-02-27-20-25-25-279 | S1-0432-08-5024_v1 | MSI-High | 27.98 | 82806 | OCAPlus_20210121.msiControl.json | No warning |

For research use only. Not for use in diagnostic procedures

# OCA Plus Results

| case | MSI | TMB | LOH | MAPD | MSS | TMB | Gene | chr | position | Exon | cDNA | protein | NM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5036 | 6.09 | 6.66 | **68.08** | 0.338 | MSS | low | BRCA2 (class 5) | 13 | 32913269 | 11 | c.4777G>T | p.E1593* | NM_000059.3 |

Tumor Mutational Burden (Mutations/Mb): 13.24

TMB

QC Metrics

**Average Coverage:**2998.0

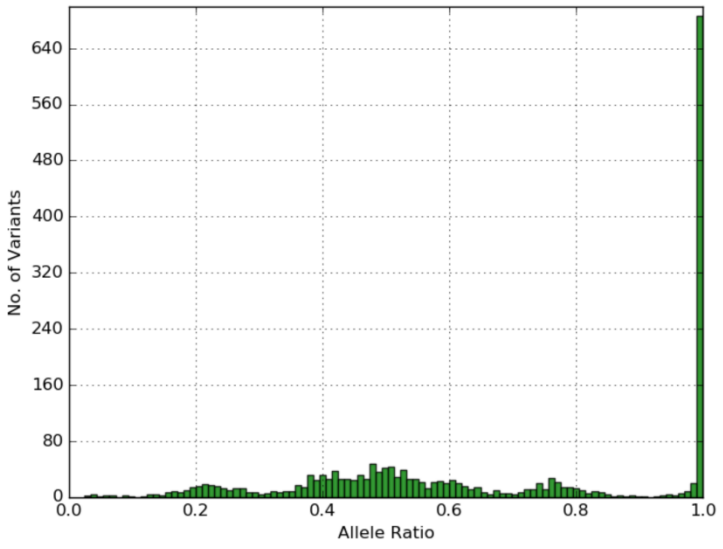
**Number of bases used in calculating TMB:** 1057691

**Number of variant calls:** 14

**TMB classification (based on specified parameters):** Undefined

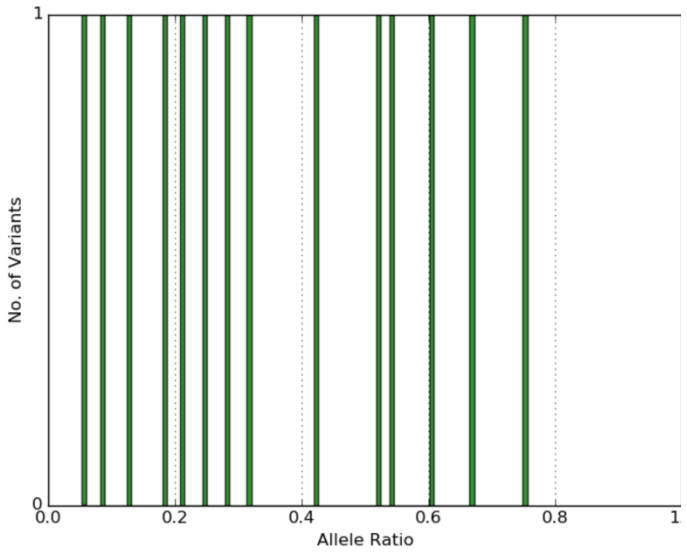
**Deamination score:** 1 (QC: PASS; observed (1) < threshold (60.0))

Germline and Somatic Variants



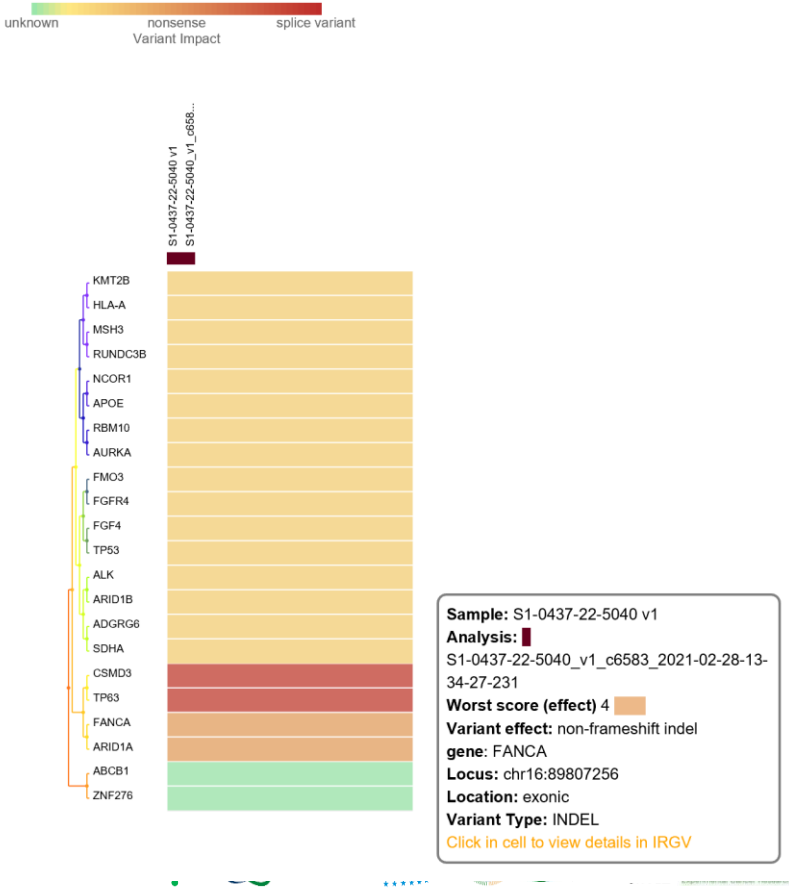

**Total Germline and Somatic Variants: 1956**

Only Somatic Variants




**Total Somatic Variants: 14**

Nonsynonymous: 12; Synonymous: 0



# Summary

- Signatures are *next generation* biomarkers indicating a functional loss of certain pathways, esp. DNA-repair mechanisms

- TMB (tumor mutation burden)
- MSI (microsatellite instability)
- HRD (homologous recombination DNA-repair deficiency)
- More to be come (Alexandrov signature)

⇒ Need for comprehensive (NGS-based) multifactorial

- Extent of effects of NEHJ/ non HRR is systematically comparable (LOH ~ LOH)

  *- Coarse granularity of NHEJ*

- Amplicon based systems have advantages when it comes to small(er) tissue samples
- OCA plus is a very reasonable solution for genomic profiling when comprehensive view is required: small genomic changes (SVV, del, ins, delins), translocations/ fusion, signatures (LOH, MSI, TMB)

# Thank you very much for your audience!