Achieving clinical confidence in Homologous Recombination Deficiency diagnostics by shallow WGS

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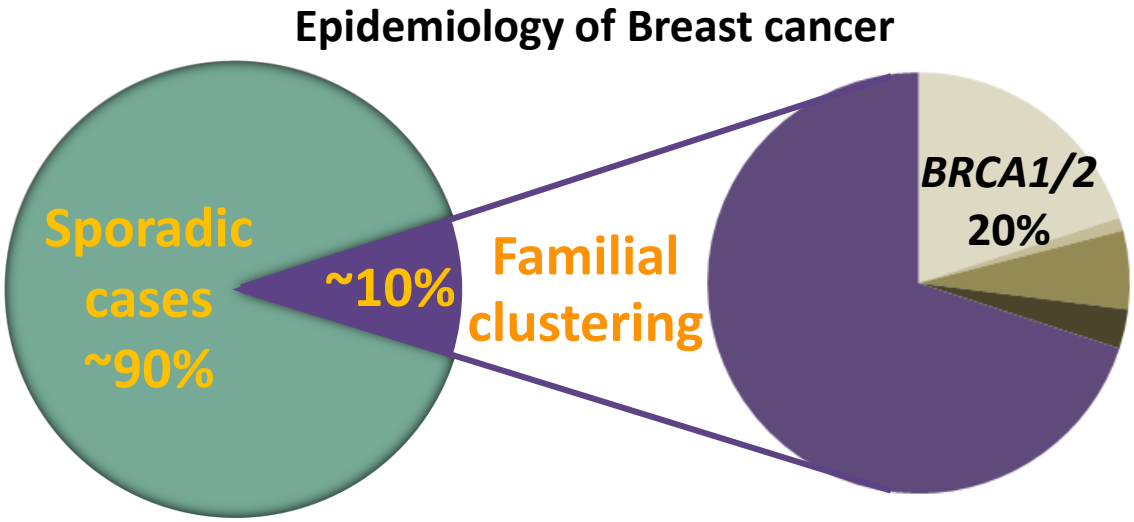
Germline deleterious mutations in BRCA1 or BRCA2 genes predispose to Breast and Ovarian cancers



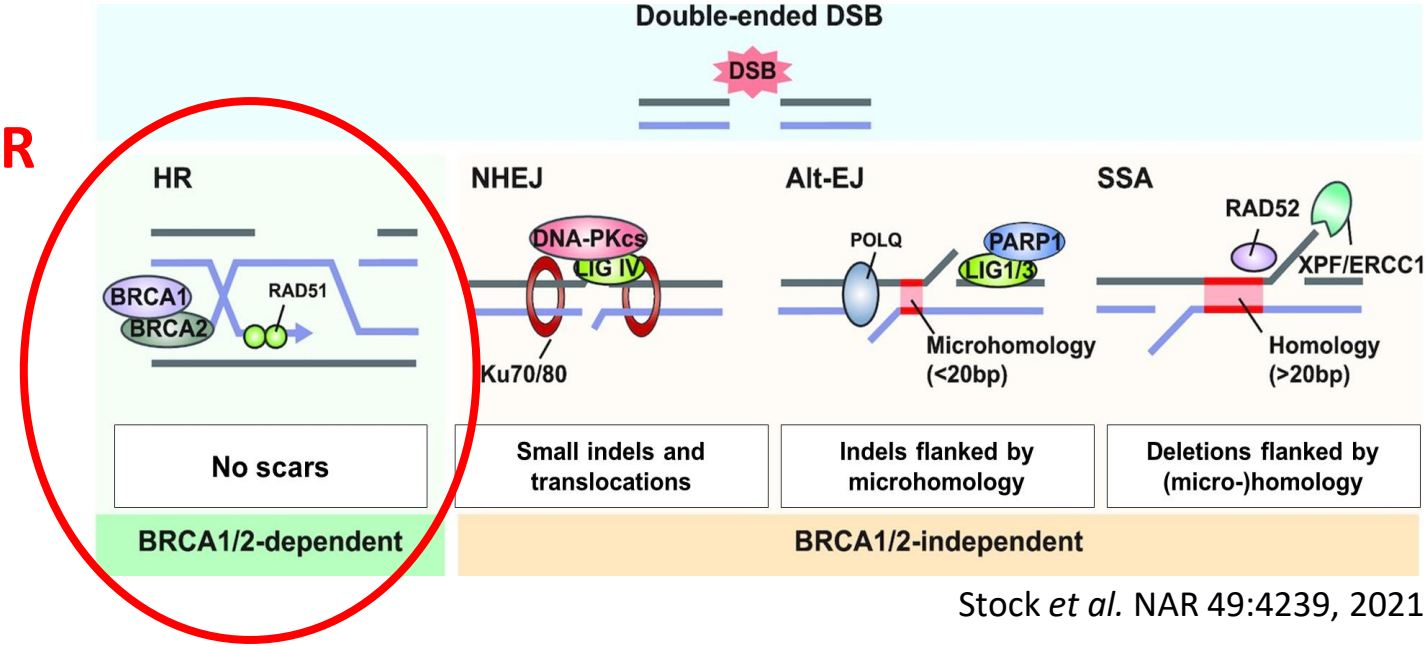
! Inactivation of BRCA1 or BRCA2 genes are frequent in Breast and Ovarian cancers

! It was not clear, how many “Sporadic” cases were driven by inactivation of BRCA1 or BRCA2

! It was clear that response to treatment is somehow different in germline mutated cases

BRCA1 and BRCA2 are major players in Homologous Recombination (HR) pathway

HR

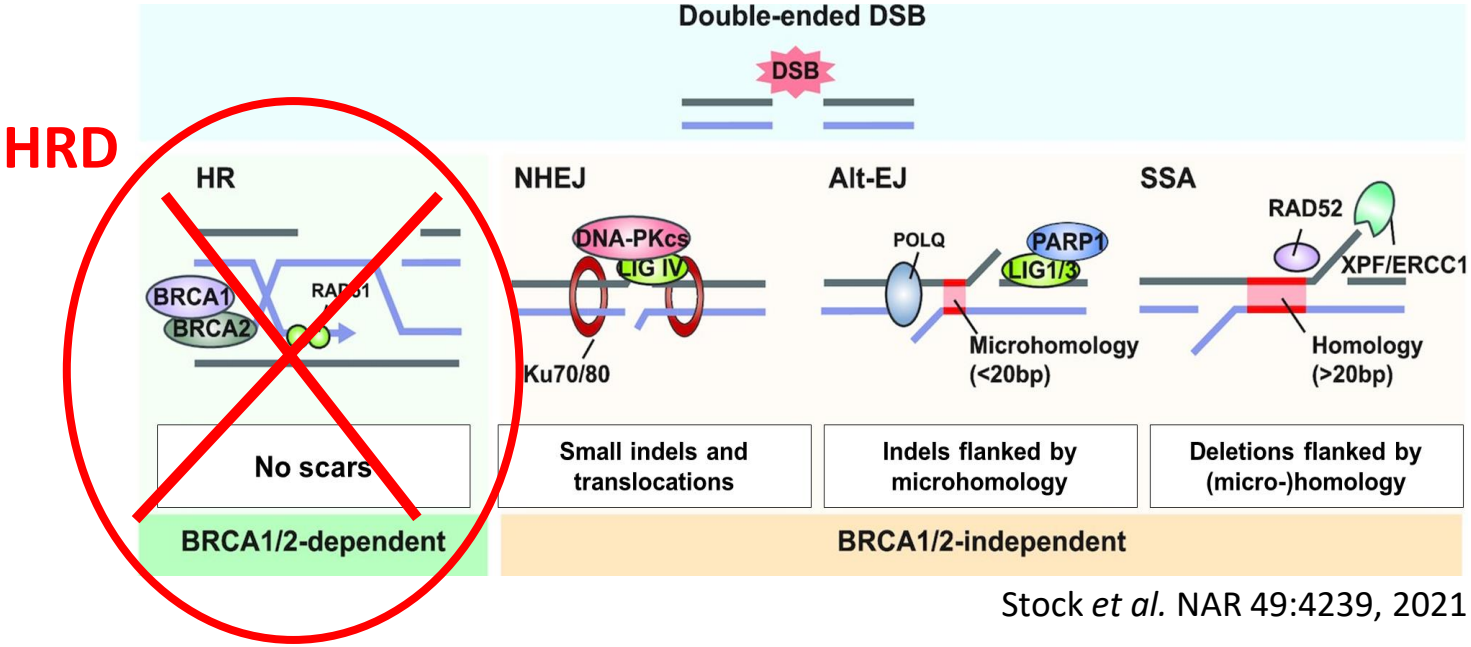


Stock *et al.* NAR 49:4239, 2021

! HR pathway **CORRECTLY** repairs double strand breaks, which are frequently arising during DNA replication

! Other pathways introduce **ERRORs** when repairing DSB (indels or structural rearrangements)

Homologous Recombination Deficiency is characterized by genomic instability

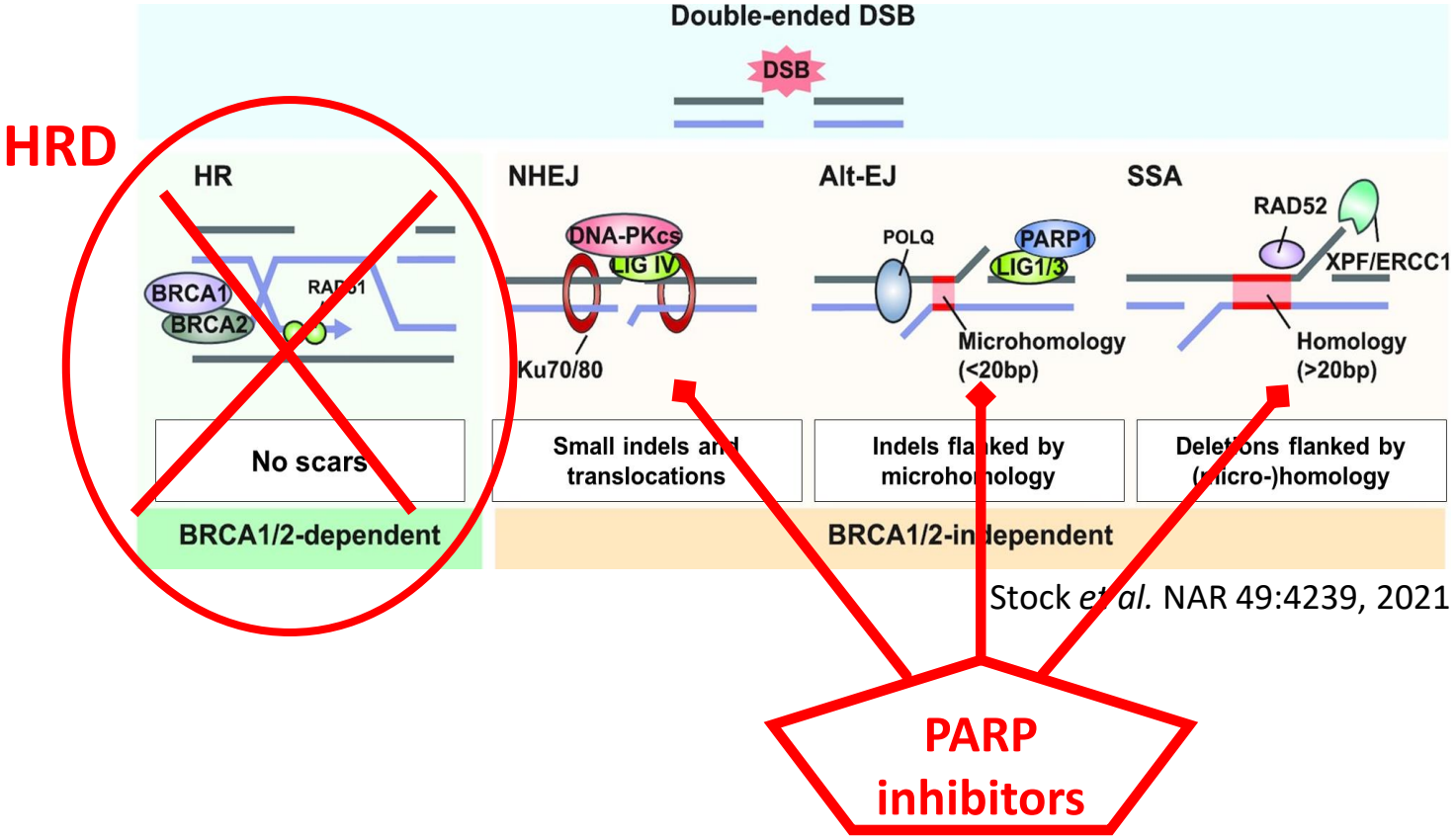


Stock *et al.* NAR 49:4239, 2021

! When Homologous recombination pathway is impaired, Double Strand Breaks are repaired by other pathways, which results in numerous **genomic alterations**

! When we sequence a tumor genome we observe these alterations at any level and named it **genomic scar**

PARP inhibitors are synthetically lethal to HRD

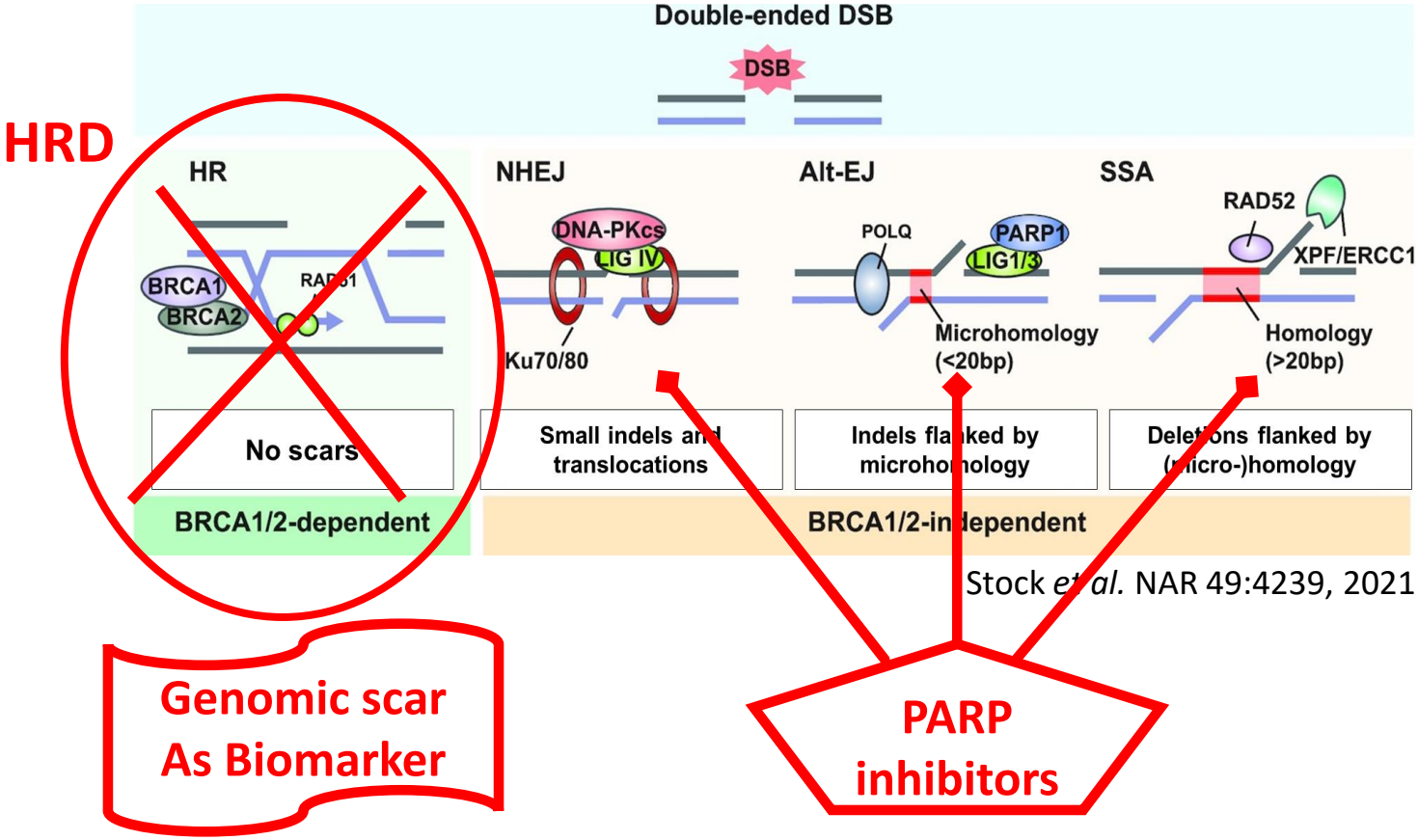


Stock *et al.* NAR 49:4239, 2021

! Homologous recombination deficiency makes tumor more sensitive to a number of DNA damaging agents (**cisplatin**) and **PARP inhibitors**, innovative drugs targeting alternative repair pathways

! Under PARP inhibitors tumor cell got overwhelmed with not repaired breaks

HOT topic for clinical application: BIOMARKER - TREATMENT



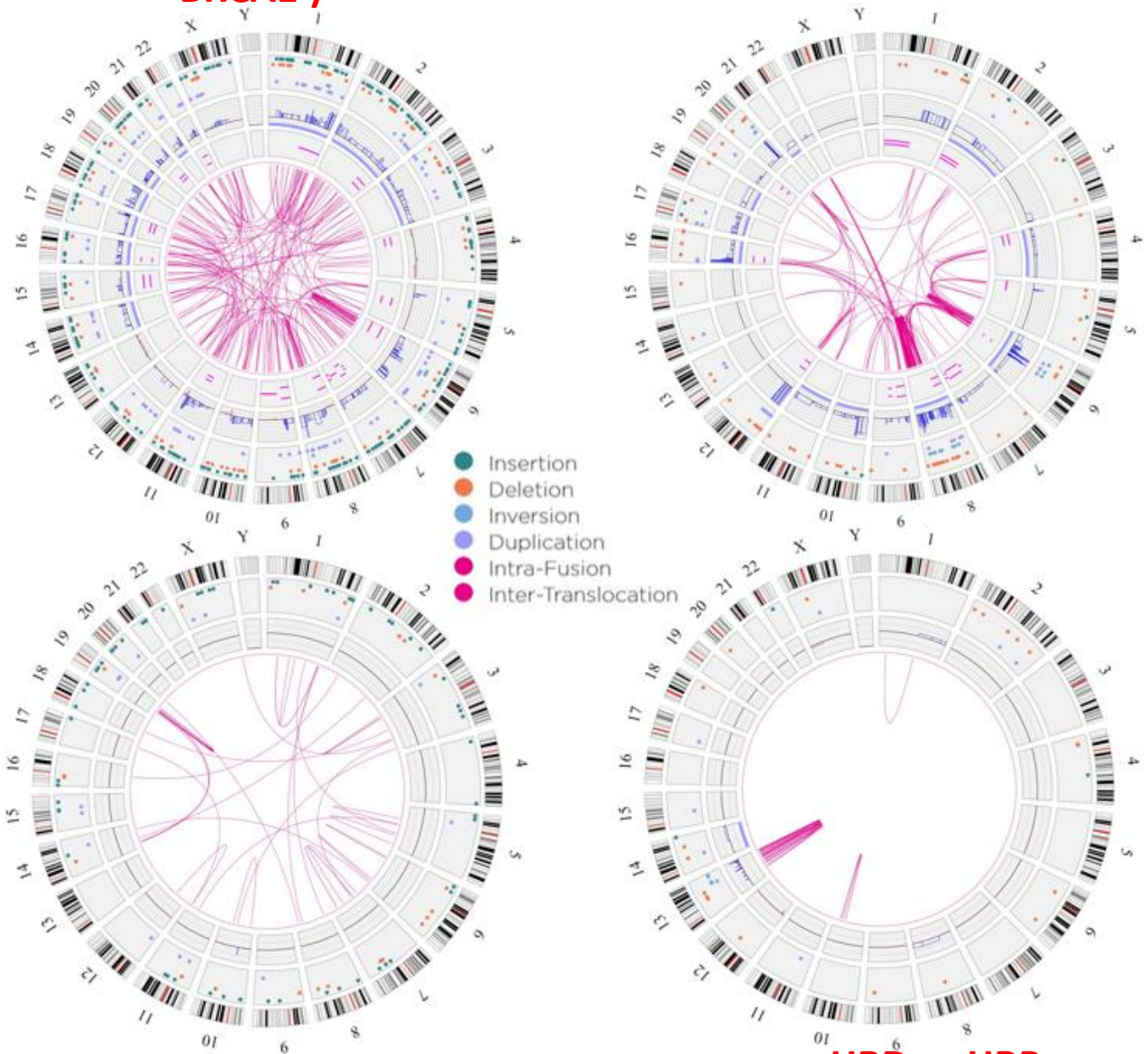
Stock *et al.* NAR 49:4239, 2021

! Many options to formalize and measure genomic scar of HRD

Biological truth: detailed genomic scar from WGS

BRCA1-/-

BRCA1/2 WT

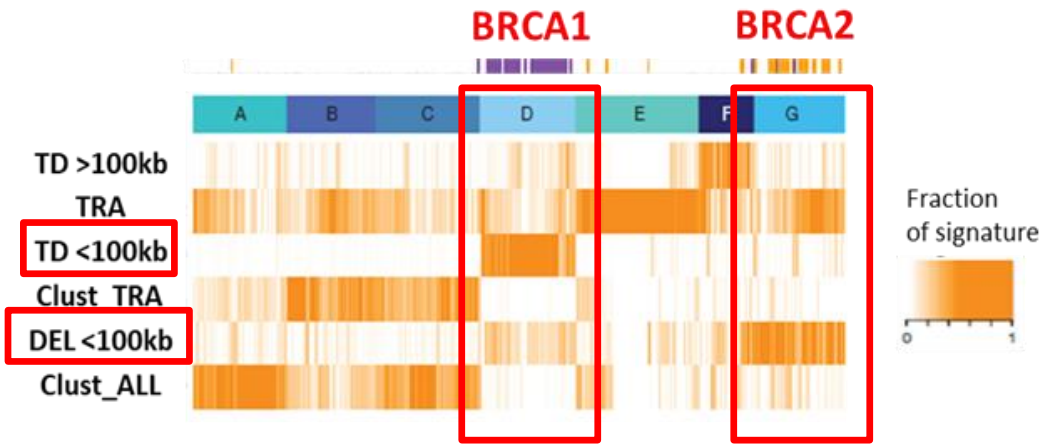


HRD or BRCAness

nonHRD or HRP

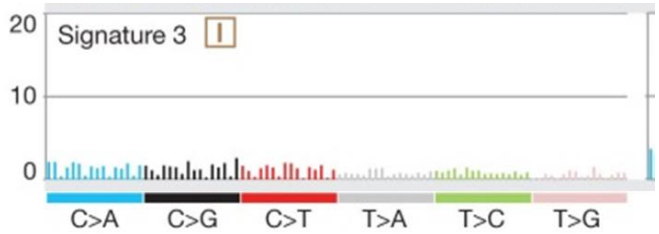
Genomic scar in HRD tumors is really highly specific

Large-scale structural alterations Breast tumors



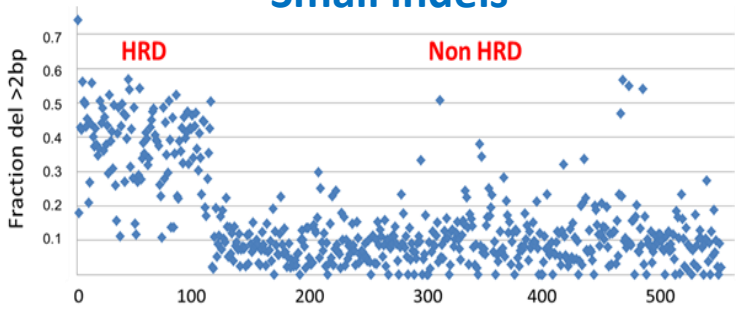
Davies H et al Nat Med. 2017
Nik-Zainal et al Nature. 2016

Single base substitutions



Alexandrov *et al. Nature* **2013**

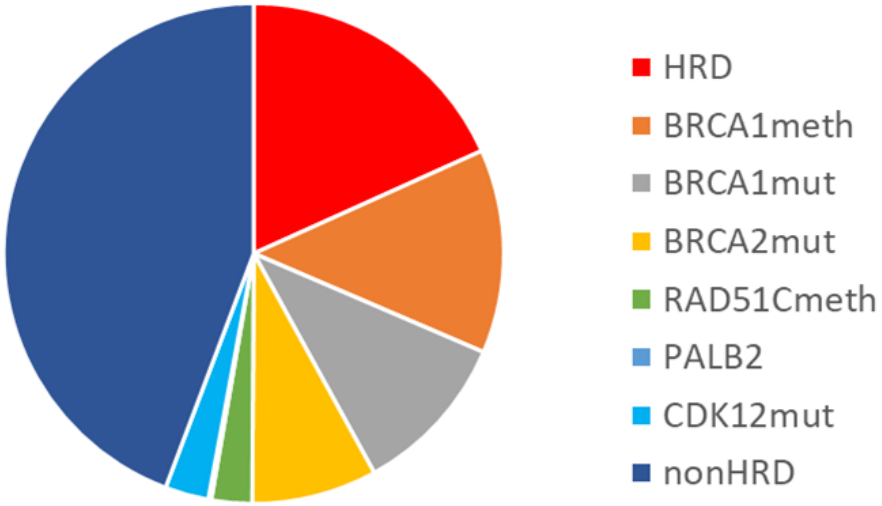
Small indels



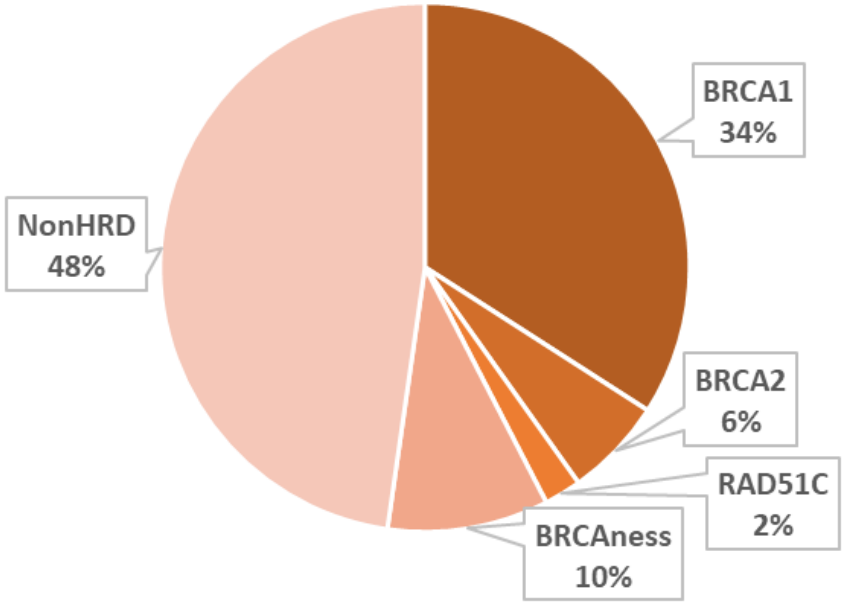
Any of these genomic features could be used for detection of tumors with HRD

Genomic analysis and clinical studies revealed all major causes of HRD in ovarian and breast cancers

Serous Ovarian Carcinoma (OvCA)

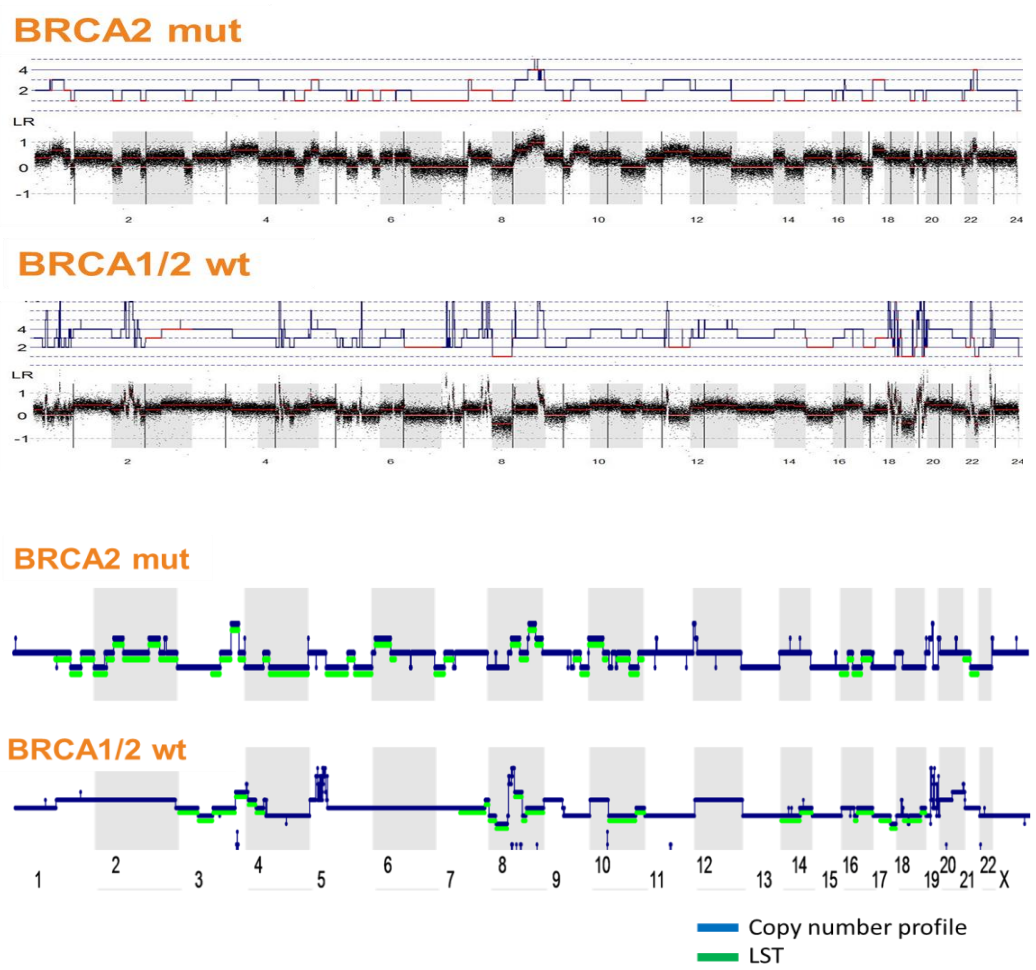


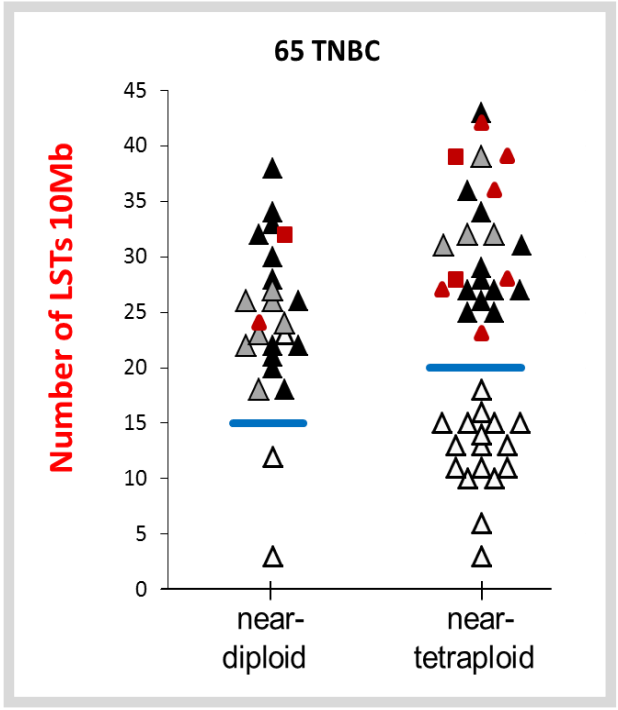
Triple-Negative Breast Cancer (TNBC)



! NEED for BIOMARKER because of multiple causes of HRD in cancers and rather high proportion of cases with “undetected” HRD origin

Our ancient Genomic signature of HRD based on Copy Number Profile





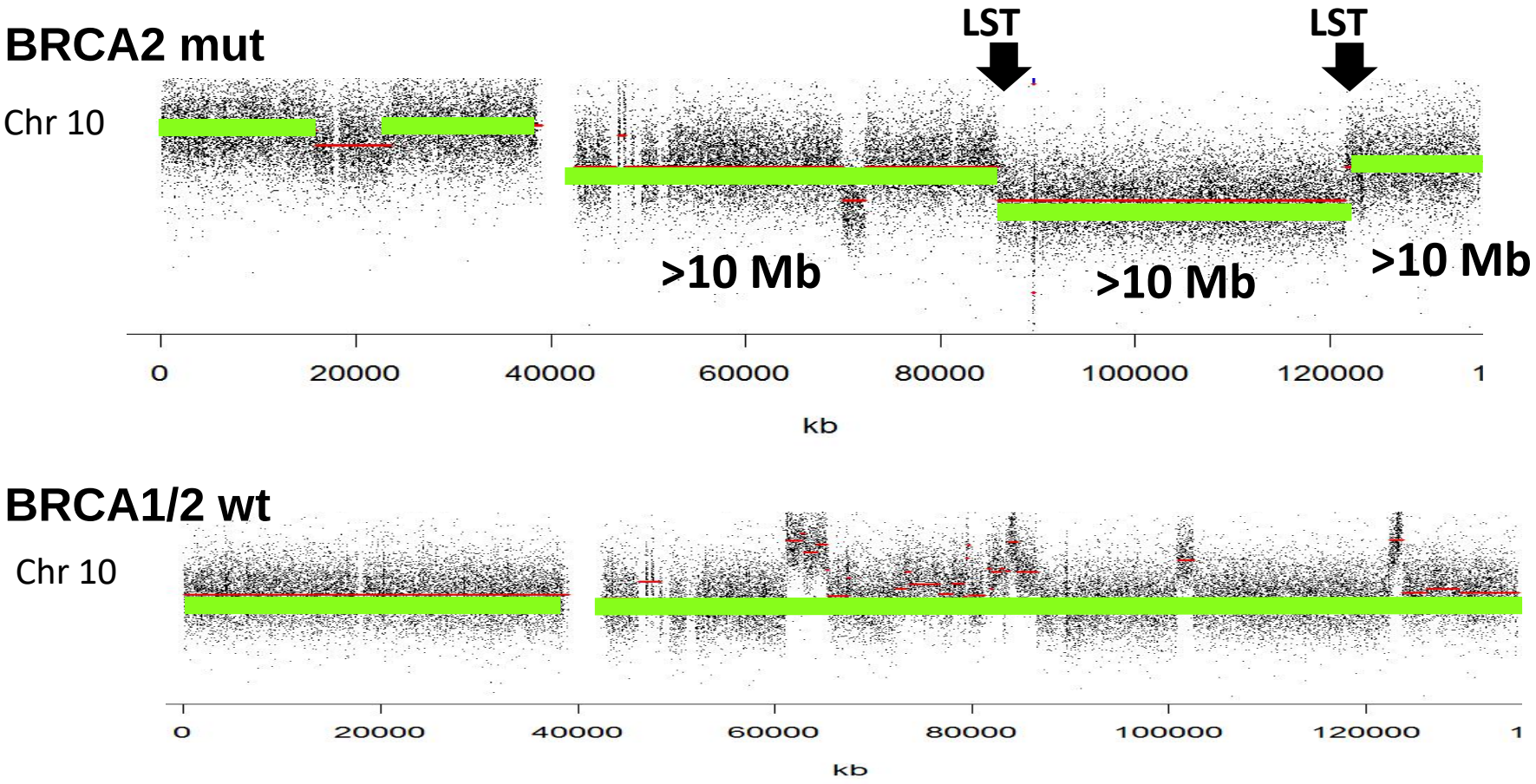
! TWO KEY IDEAS:

1) to calculate the number of LARGE-SCALE copy number breaks (named **LST**)

2) consider near-diploid and near-tetraploid cases separately (**2** cut-offs)

PATENTED and SOLD to Myriad Genetics (US) in 2012!

For note: Largescale State Transitions (LST) copy number breaks between LARGE segments



! LST - chromosomal breaks between segments of >10Mb in size after filtering small (<3Mb) alterations

That's all history ☺

HRD TEST of Myriad Genetics

Telomeric Allelic Imbalance Indicates Defective DNA Repair and Sensitivity to DNA-Damaging Agents

Nicolai J. Birkbak, Zhigang C. Wang, Ji-Young Kim, et al.

Cancer Discover

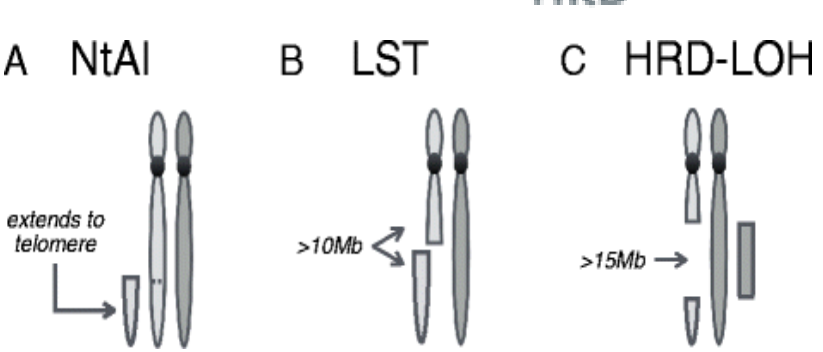
Ploidy and Large-Scale Genomic Instability Consistently Identify Basal-like Breast Carcinomas with *BRCA1/2* Inactivation

Tatiana Popova
Thierry Dubois
Xavier Sastre-Ga

Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer

V Abkevich, KM Timms, BT Hennessy, J Potter, MS Carey, LA Meyer, K Smith-McCune, R Broaddus, KH Lu, J Chen, TV Tran, D Williams, D Iliev, S Jammulapati, LM FitzGerald, T Krivak, ...

MYRIAD myChoice HRD

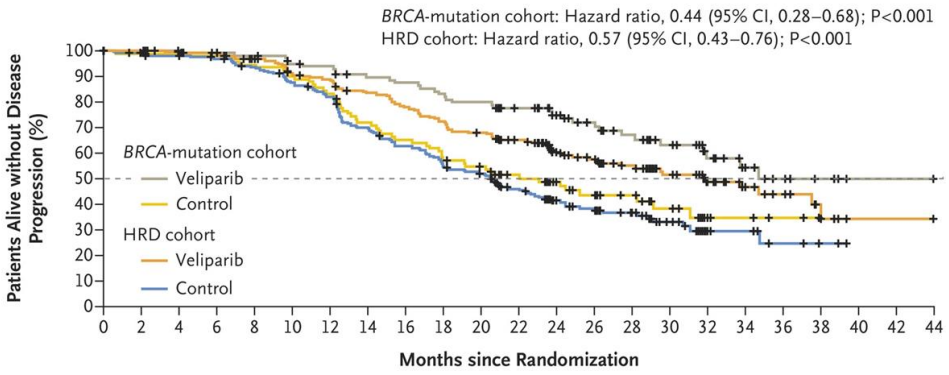


FDA approval of the HRD TEST

The NEW ENGLAND JOURNAL of MEDICINE

Coleman et al. 2019

Veliparib with First-Line Chemotherapy and as Maintenance Therapy in Ovarian Cancer



shallowHRD approach by A Eeckhoutte

Genome analysis

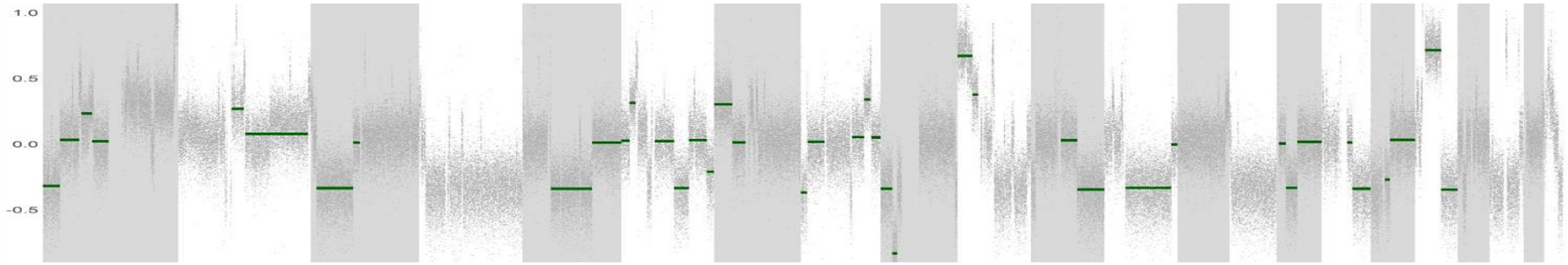
ShallowHRD: detection of homologous recombination deficiency from shallow whole genome sequencing

Alexandre Eeckhoutte [1,2,*], Alexandre Houy[1,2], Elodie Manié[1,2], Manon Reverdy[1,2], Ivan Bièche[3], Elisabetta Marangoni[2,4], Oumou Goundiam[2,4], Anne Vincent-Salomon[5], Dominique Stoppa-Lyonnet[1,6], François-Clément Bidard[7,8], Marc-Henri Stern[1,2,3] and Tatiana Popova[1,2]

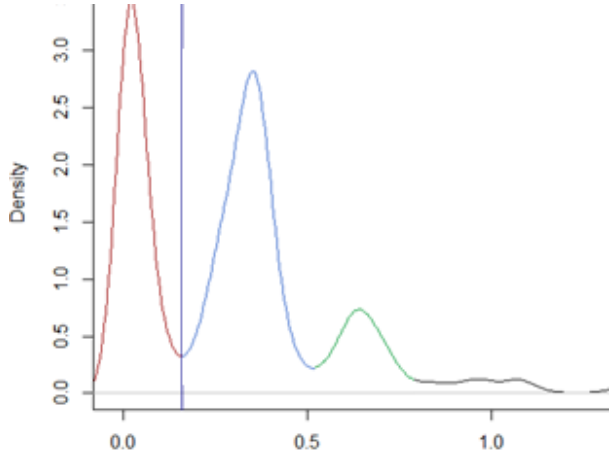
shallowHRD approach: cheap and simple test for HRD

Alex Eeckhoutte

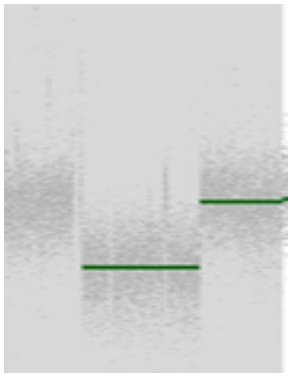
Shallow WGS ~1X coverage, CNA profile and Large Genomic Alterations (LGA)



CN break cut-off



LGA counts

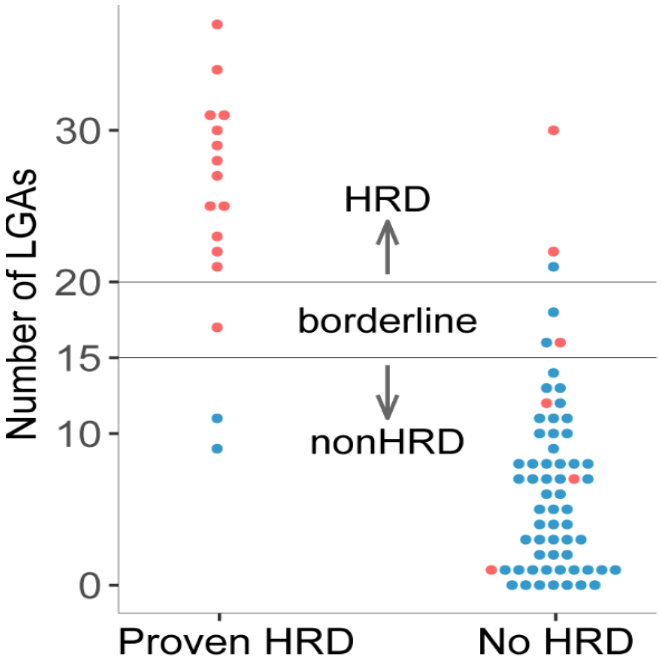


shallowHRD was well appreciated in Curie clinical research

shallowHRD approach: cheap and simple test for HRD

Alex Eeckhoutte

Decision rule



Performance

Technique	Method	Sensitivity	Specificity
WGS	HRDectect	99	99
WGS/WES	Signature 3	84	90
WES/gene panel	SigMA	74	90
WES	scarHRD	87.5	61.4*
SNParray	LST	99	54*
sWGS	shallowHRD	87.5	90.5

Eeckhoutte et al Bioinformatics 2020

~70% of cases are correctly segmented and LGA corresponds to genomic profile
~30% gave inconclusive results or false diagnostics

For clinical application shallowHRD needed improvements!

Three major problems in HRD testing with shallow WGS

I. Sequencing quality is out of control: FFPE samples are often very noisy
sWGS + Dragon technic does not allow controlling coverage

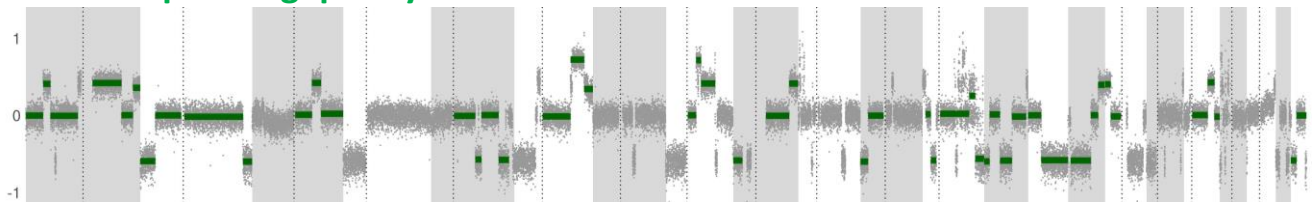
II. NO ground truth available for all cases neither for HR genes inactivation nor for Copy number alteration profile: Scarce annotation of HRD cases does not allow automatic supervised classification

III. Borderline scores: Varying Breast and Ovarian cancer genome complexity + compromised quality is producing many un-decisive diagnostics

Business plan: Develop shallowHRD_v2 with quality control and refined diagnostics!

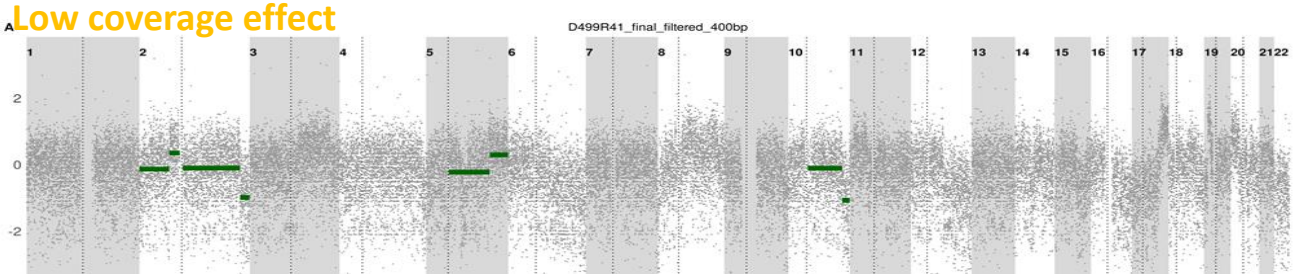
I Sequencing quality: typical outcomes for shallow WGS

Good sequencing quality Fresh Frozen FF



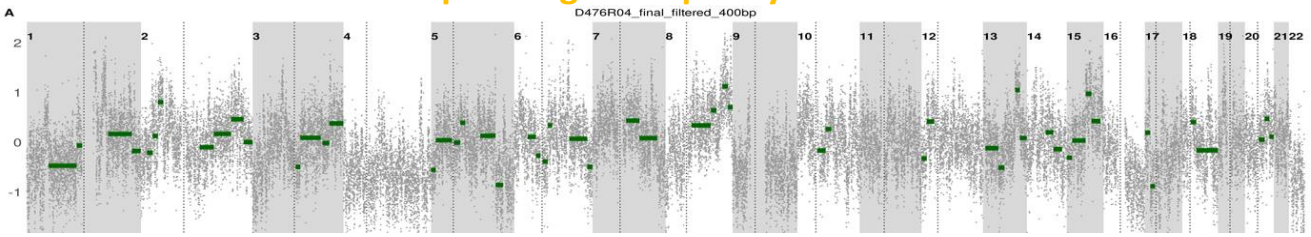
>0.5X coverage

Low coverage effect



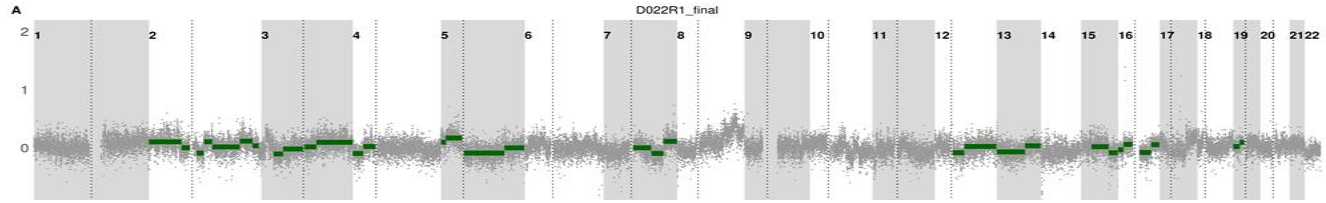
<0.1X coverage

Clinical FFPE affect the sequencing DNA quality



30% of FFPE are affected

Low tumor content

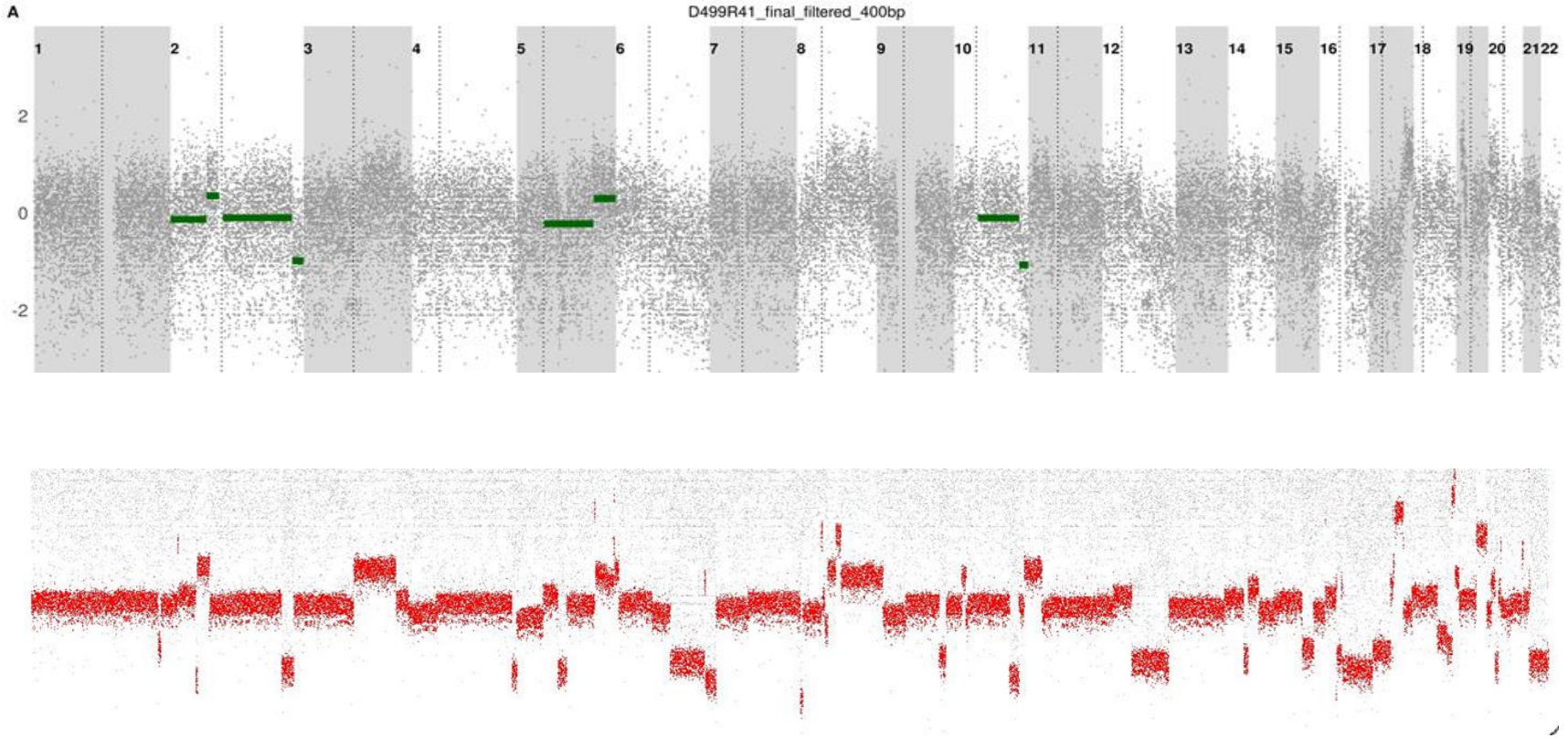


5% not possible to use
10% possible to use but…

Solution: Profile calibration, noise correction and quality categorization

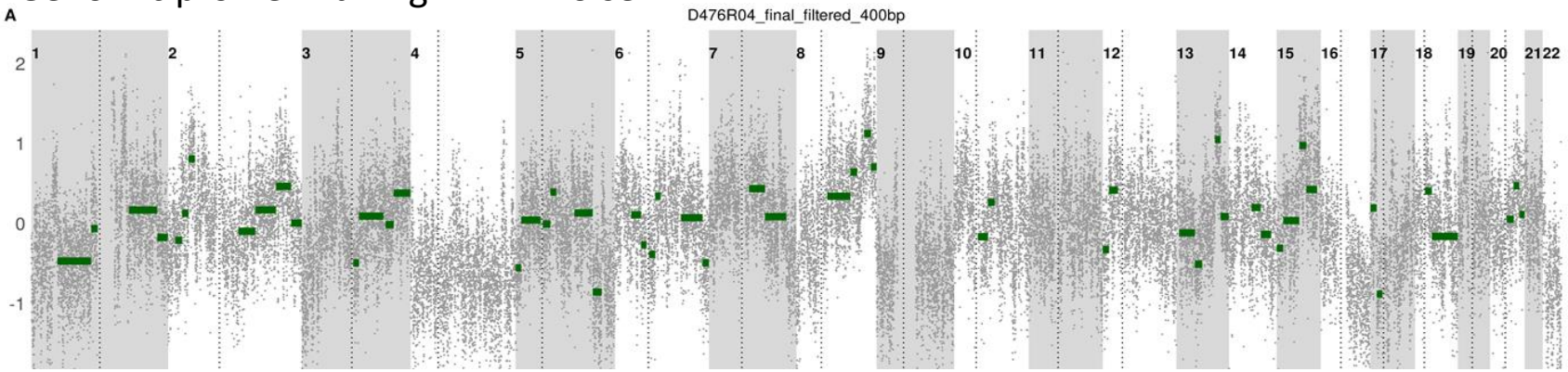
1 “Standardization” of CNA profile

Normalization of the profile to standardized raw variance

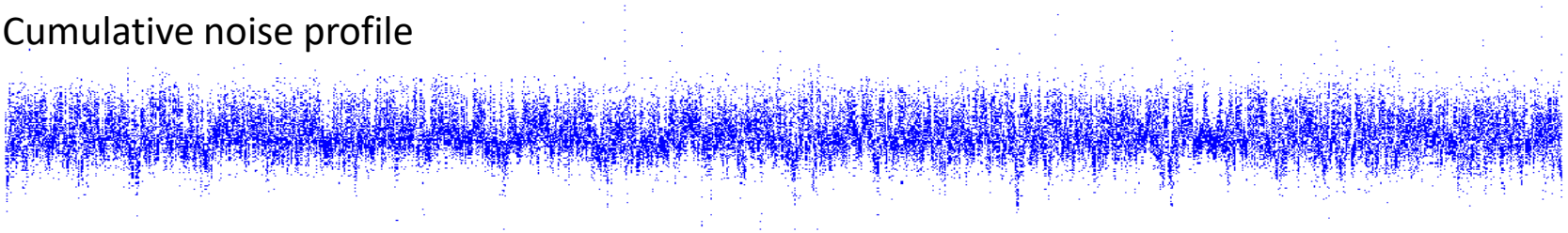


FFPE noise is systematic and not depends on GC

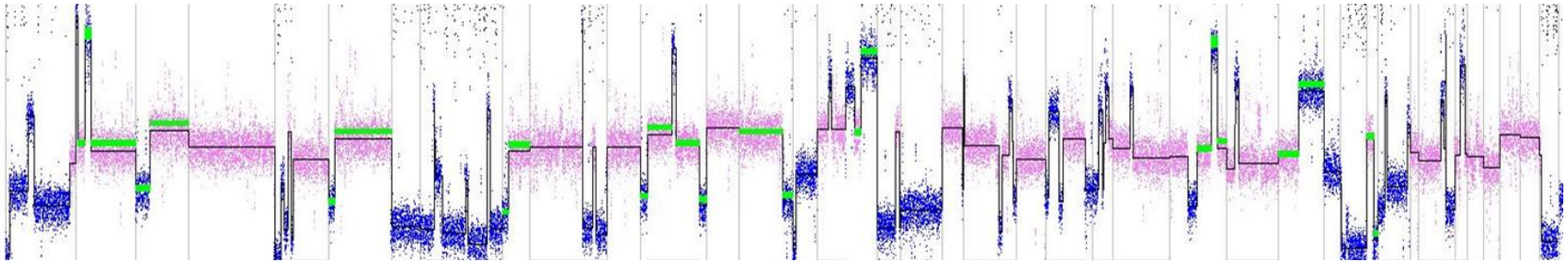
Genomic profile with high FFPE noise



Cumulative noise profile



Corrected profile



3 Categorization of samples by QUALITY

Classification of the profiles based on quality and tumor content

Profile segmentation and characterization
- N breakpoints
- Variance total
- Variance within segment,
- Variance between segments
- Correlation to FFPE noise
- etc

Profile classification into the groups

Raw variance (variance within the segment):
0 – low,
1 – increased,
2 – high

Tumor content (variance of medians of the large segments):
0 – no tumor,
1 – low,
2 – average,
3 – high

FFPE noise (N_bp, correlation to FFPE, variance of error profile):
0 – no FFPE covariate
1 – low,
2 – increased,
3 – high

Optimization of the CNA profile and updating quality categories

Selection of the **adaptive threshold** for between segment difference to be considered as negligible

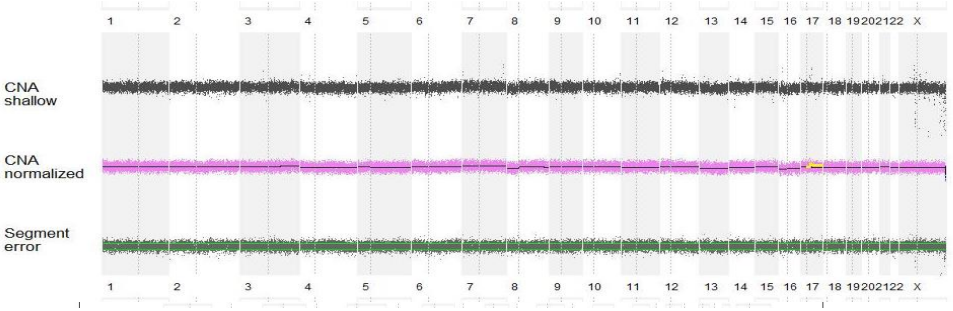
Profile optimization:
Uniting the adjacent segments if the median difference is less than a **threshold**

Profile correction:
Eliminating the breakpoint(s) if it (they) follows the breakpoint in FFPE covariate profile even if the difference exceeds the threshold

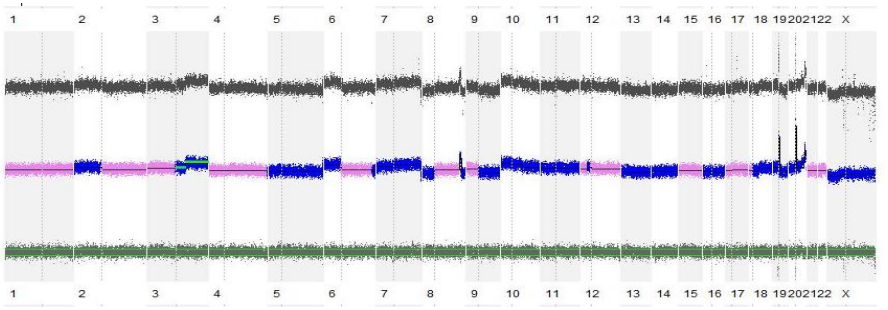
Updating quality categories if the case

Examples: Visual and automatic control by Error profile

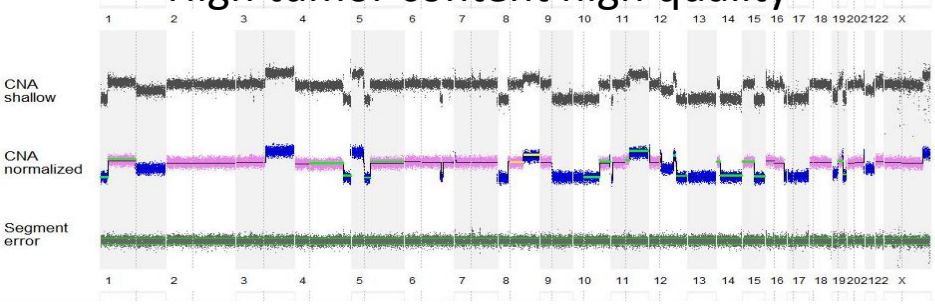
No tumor high quality



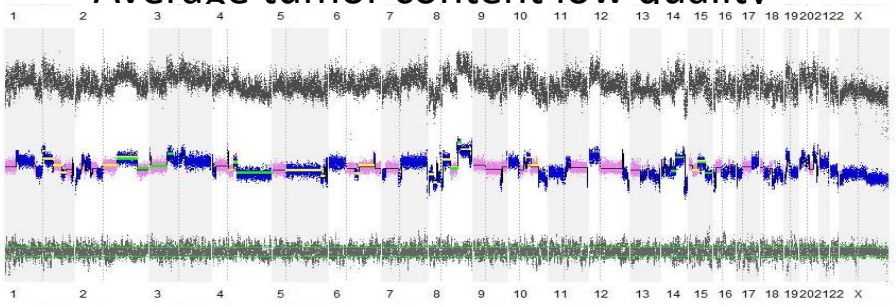
Low tumor content high quality



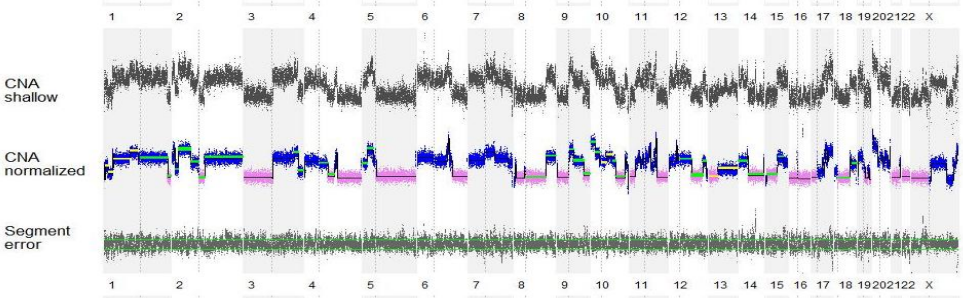
High tumor content high quality



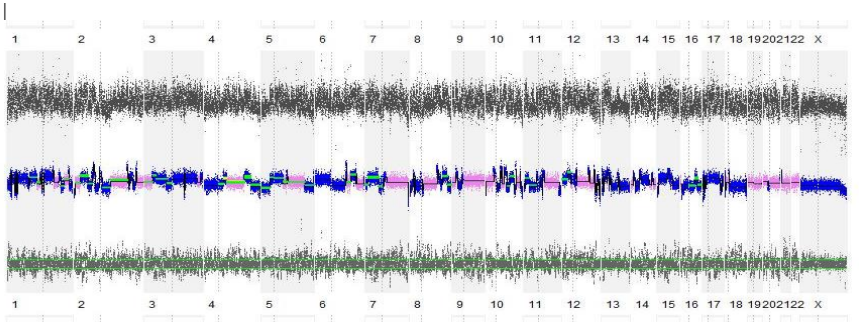
Average tumor content low quality



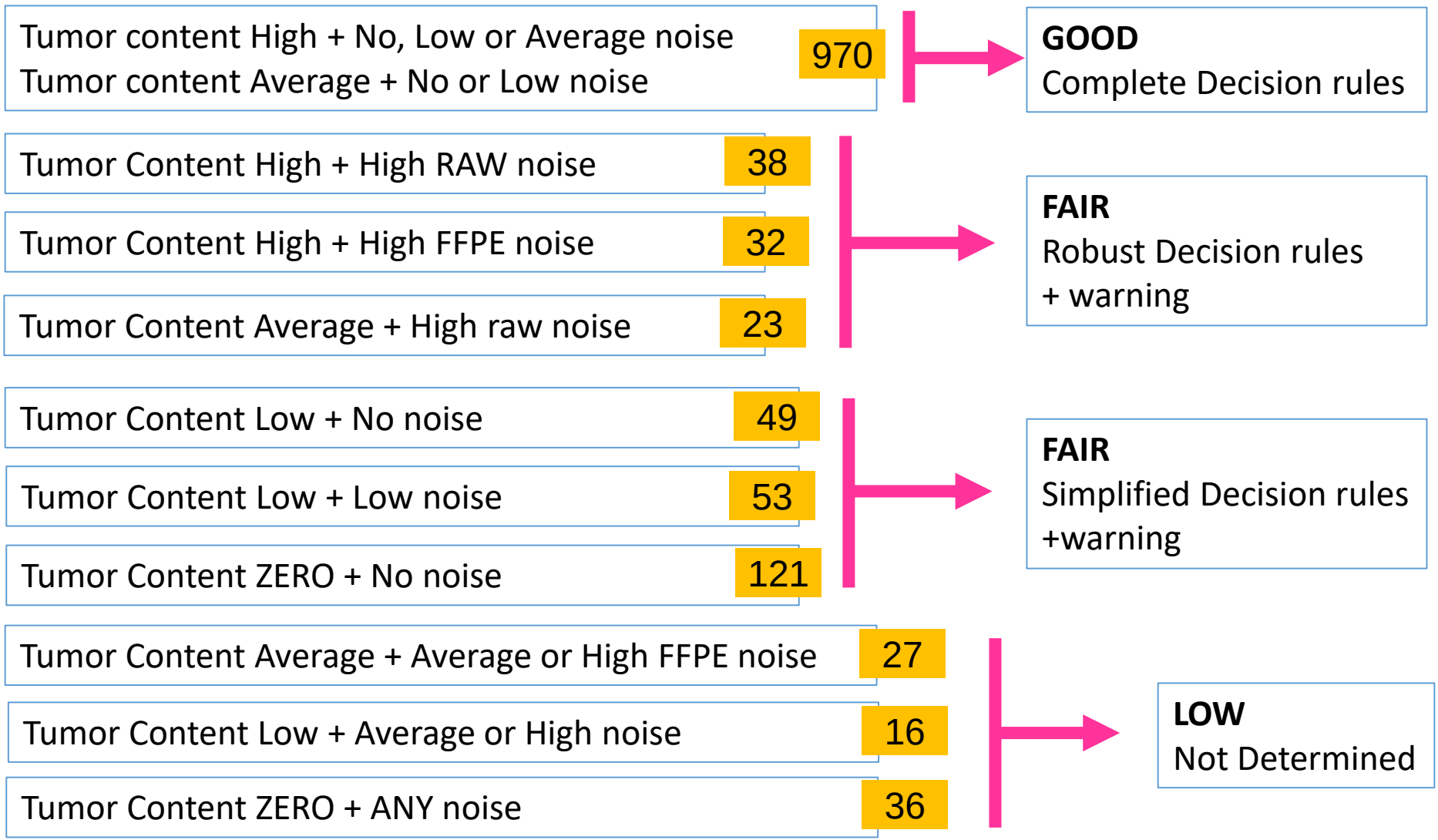
High tumor content average quality



Low tumor content low quality



II. Training set quality clusters and annotation by expert

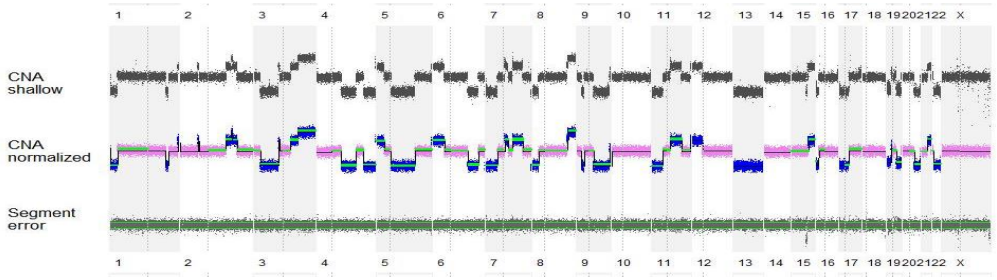


II. Pattern recognition in GOOD cases

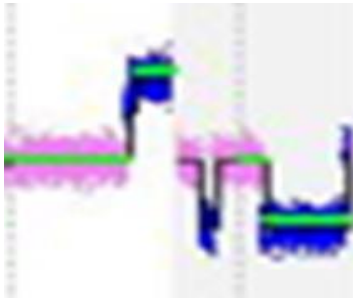
"Pattern recognition according to IQ test designers is a key determinant of a person's potential to **think logically, verbally, numerically, and spatially.** Compared to all mental abilities, **pattern recognition is said to have the highest correlation with the so-called general intelligence factor"** (Kurzweil, 2012). Jan 18, 2018, www.psychologytoday.com

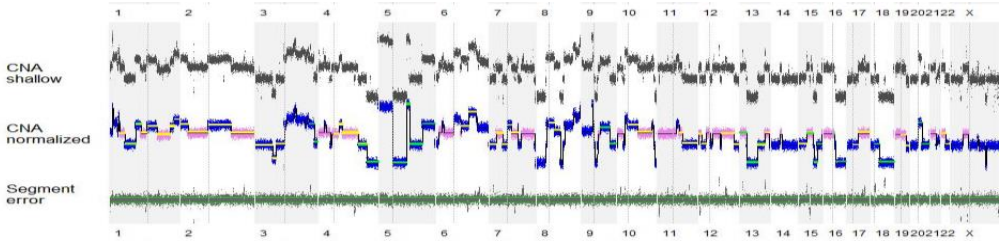
7 Manual classification into HRD/nonHRD etc.

Focus on the GOOD cases and **L**arge**G**enomic**A**lteration number

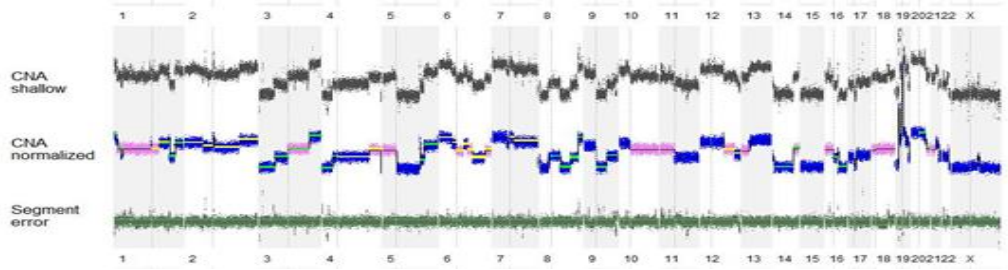


HRD “SIMPLE”
genome
LGA<20





HRD “COMPLEX”
genome
LGA>20



nonHRD “COMPLEX”
CCNE1 amplify
20<LGA<25

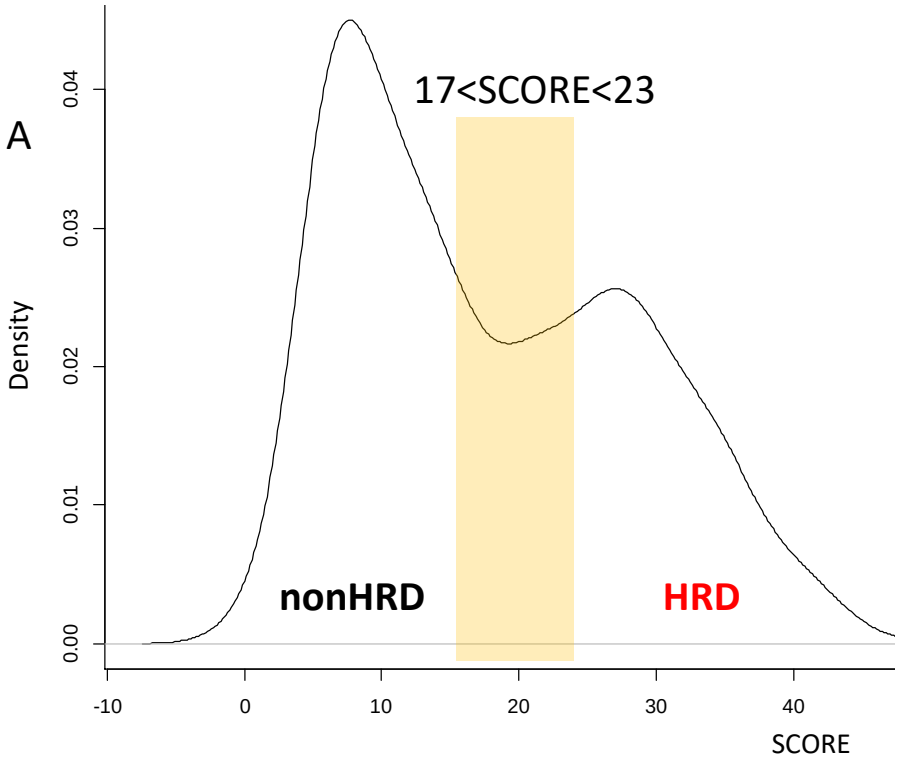
The IDEA was to modify LGA number using PENALTY and BONUS
PENALTY if some feature is frequent in nonHRD
BONUS if some feature is frequent in HRD

8 Parameters for PENALTY and BONUS

Formalization CNA phenotypes

PENALTY	BONUS
Amplification of **CCNE1** or **HER2**	Detecting the **baselines**: **max2CN** = load of 2 most abundant CN levels
The number of interstitial gains and deletions for **CDK12**mut call	Estimating genome **complexity**: SIMPLE if **max2CN** ≥ **0.7** COMPLEX if **max2CN** < **0.7**
Amplification pheno: N chr arms with amplification ≥ 3	**HighCN** = number of CN levels with significant load COMPLEX+ if **HighCN** ≥4
PENALTY = 5 if Amplif phenotype, CCNE1/HER2 amplif or CDK12mut **PENALTY=8** if any 2 features	**BONUS = 5** If SIMPLE

SCORE distribution in the TRAINING set

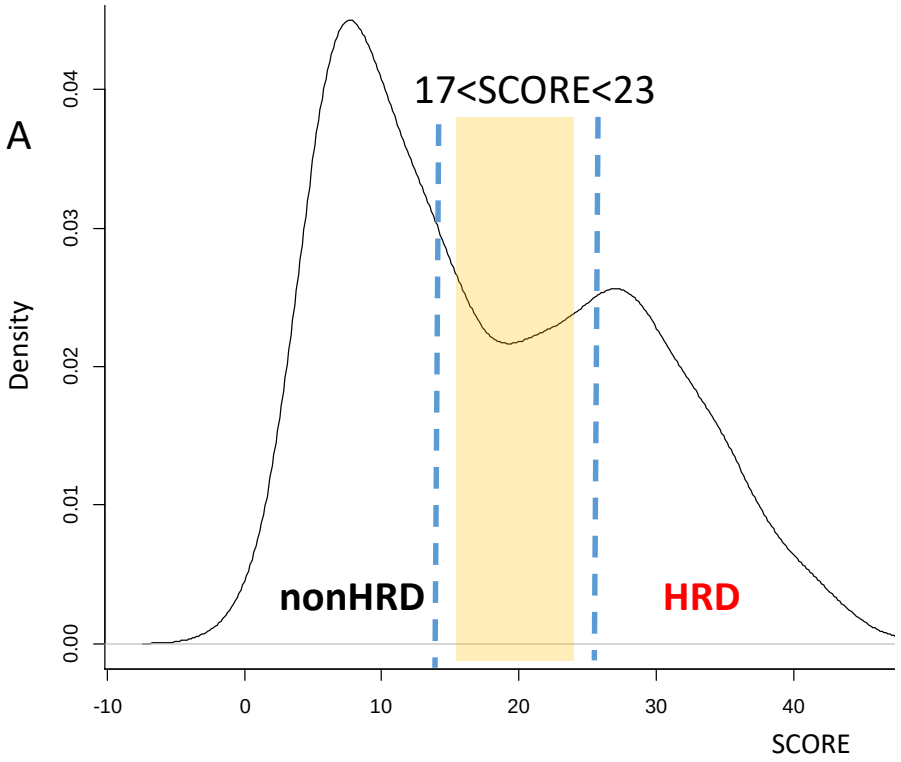


SCORE = LGA – PENALTY + BONUS

~80% of cases satisfy these conditions with **100% correct** predictions in good quality samples
~20% of borderline cases need additional criteria

borderline cases include: true borderline scores, mistakes in complexity estimation, mistakes in breakpoints detection due to noise, etc

SCORE distribution in the TRAINING set

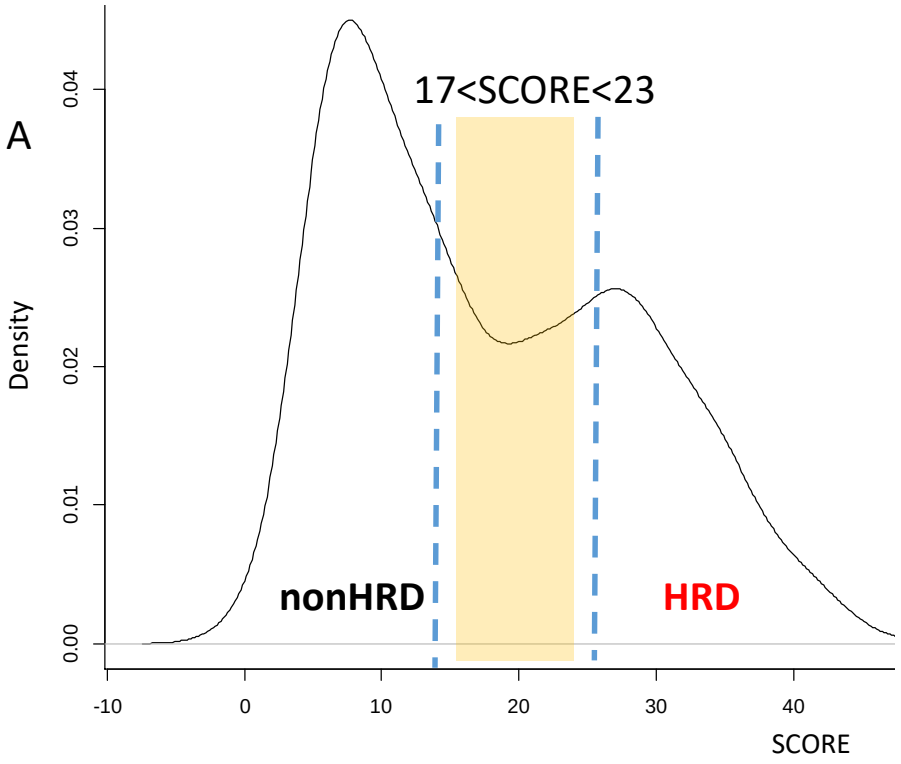


SCORE = **LGA** – **PENALTY** + **BONUS**

Random initiation of segmentation algorithm
&
stochastic process of profile optimization
&
the system of fixed thresholds
=>
possible variation in SCOREs(!)

20 segmentation/optimization runs gives SCORE and SCORE_SD defining “CLEAR-CUT” or “BORDERLINE” attribution

SCORE distribution in the TRAINING set



SCORE = **LGA** – **PENALTY** + **BONUS**

LGA

Calling LGA:
breakpoints between segments of 10Mb using 2 **adaptive thresholds**:

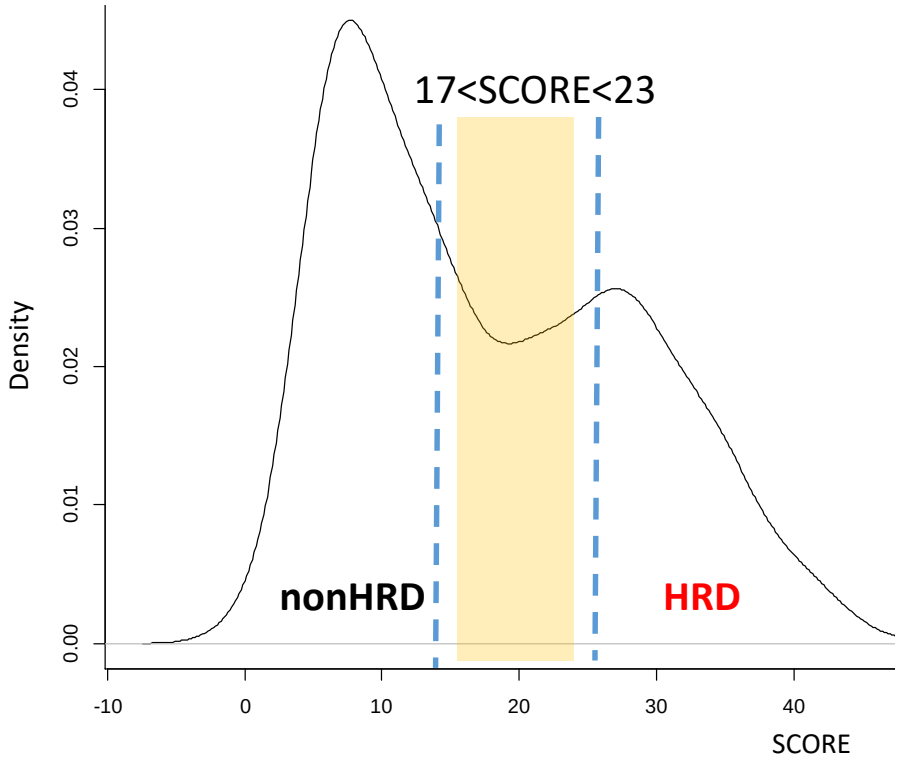
Stringent (for SIMPLE genome)
and
Soft (for COMPLEX genome)

LGA_10Mb_soft
LGA_10Mb_stringent

HRD and nonHRD sure attribution is done for less advantageous SCORE

~25% of **secure** borderline cases need additional criteria

III. Borderline scores



What kind of cases are in borderline?

1. Cases with PENALTY
2. Cases with “not even” distribution of LGA
3. Cases with higher complexity
4. Mistakes in segmentation or recognition
5. TRUE borderline

TRUE borderline:

LGA=13 BONUS=5 => SCORE=18

LGA=14 BONUS=5 => SCORE=19

LGA=19 BONUS=0 => SCORE=19

IDEA To find some additional genomic parameters, which are FAR from borderline

9 More parameters and features for borderline cases

PENALTY helps resolving some of TRUE borderline cases

PENALTY helps resolving borderline cases:

HRD call rule: **17** < **SCORE** < **23** and **PENALTY** ≥ **5** → **nonHRD**

PENALTY is defined by:
CCNE1 amplification, HER2 amplification, focal amplification affecting minimum 3 chromosome arms, CDK12mut phenotype (high number of interstitial gains)

9 More parameters and features for borderline cases

Idea of cumulative index: **LGA_boost**

LGA_boost

1. LGA_chromosome_arm: N chromosome arms with LGA
2. LGA_at_telomere: N chromosome arms with LGA involving telomeric region
3. LGA_20Mb: N of LGA 20Mb
4. LGA_baseline: N of LGA calls with the most abundant CN level (baseline)
5. LGA_baseline_12: N of LGA calls between the segments from the two most abundant CN level

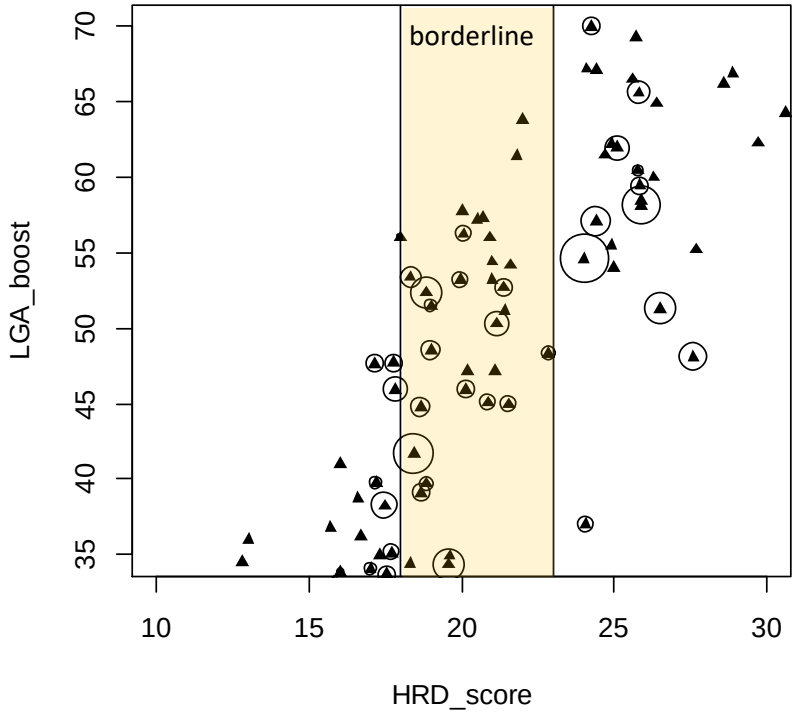
(1+2+3)_soft +4+5 = **LGA_boost**_soft

(1+2+3)_stringent+4+5 = **LGA_boost**_stringent

LGA_boost accounts for the “typical” HRD phenotype with large-scale breaks randomly distributed along the genome

9 More parameters and features for borderline cases

SCORE and LGA_boost in SIMPLE genome

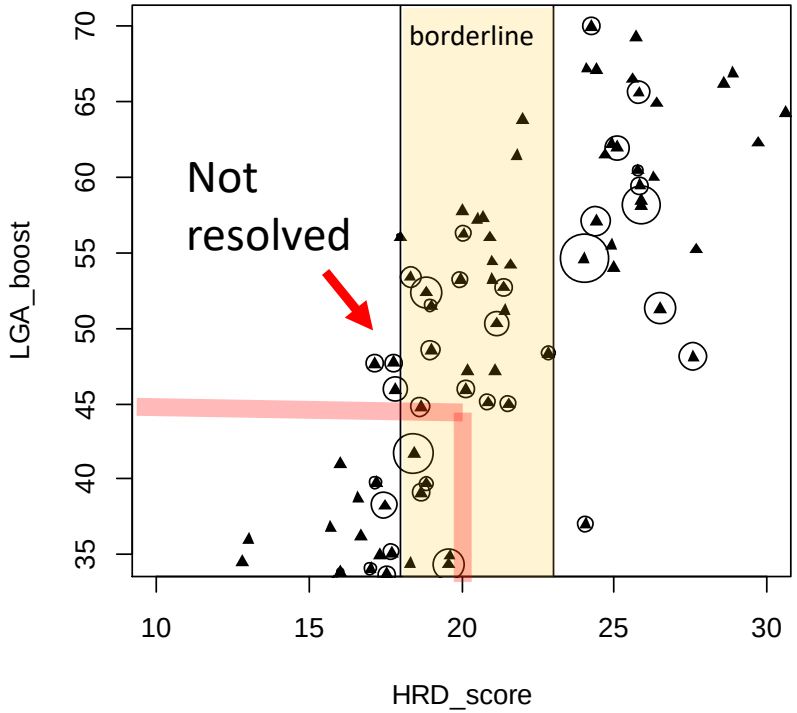


Points are tumor genomes,
Circles indicate standard errors of LGA in 20 runs

No clear VISIBLE cut-off, but some cases could be resolved!

9 More parameters and features for borderline cases

SCORE and LGA_boost in SIMPLE genome

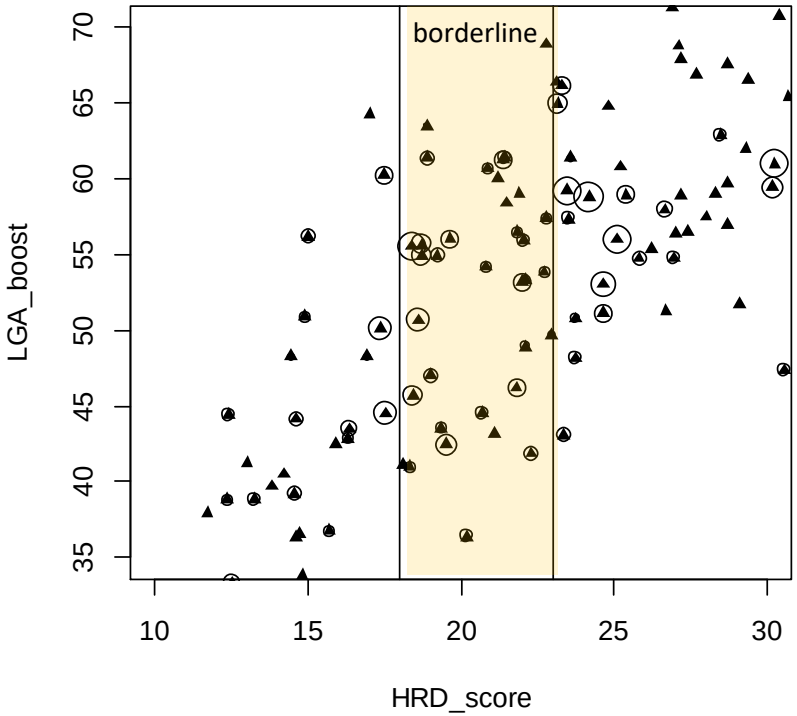


Points are tumor genomes,
Circles indicate standard errors of
LGA in 20 runs

HRD call rule: SIMPLE genomes at the borderline and
LGA_boost ≥ 45 or SCORE ≥ 20 → **HRD**

9 More parameters and features for borderline cases

SCORE and LGA_boost in COMPLEX genome

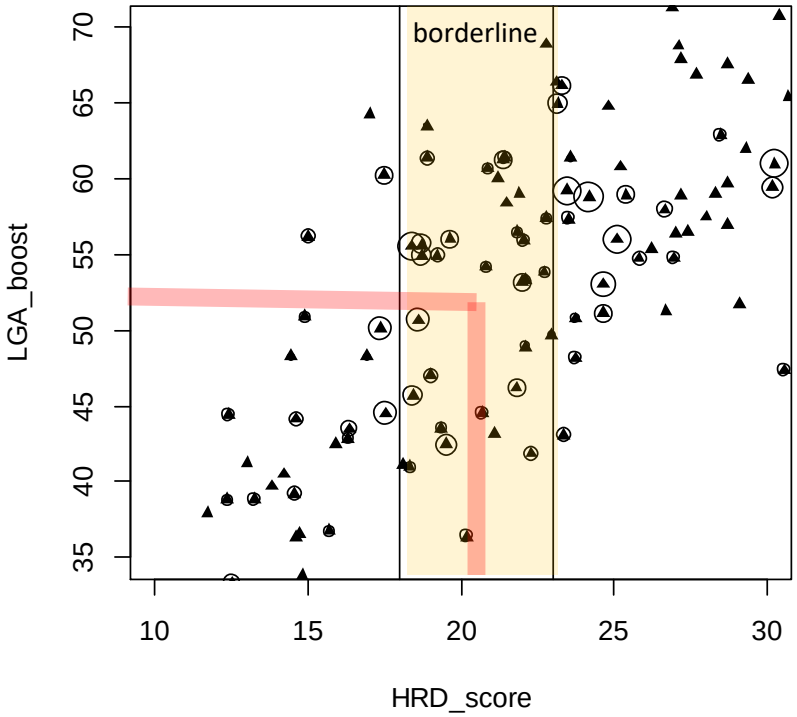


Points are tumor genomes,
Circles indicate standard errors of LGA in 20 runs

Some VISIBLE clustering, some cases could be resolved, but need more cases with annotation to optimize class separation

9 More parameters and features for borderline cases

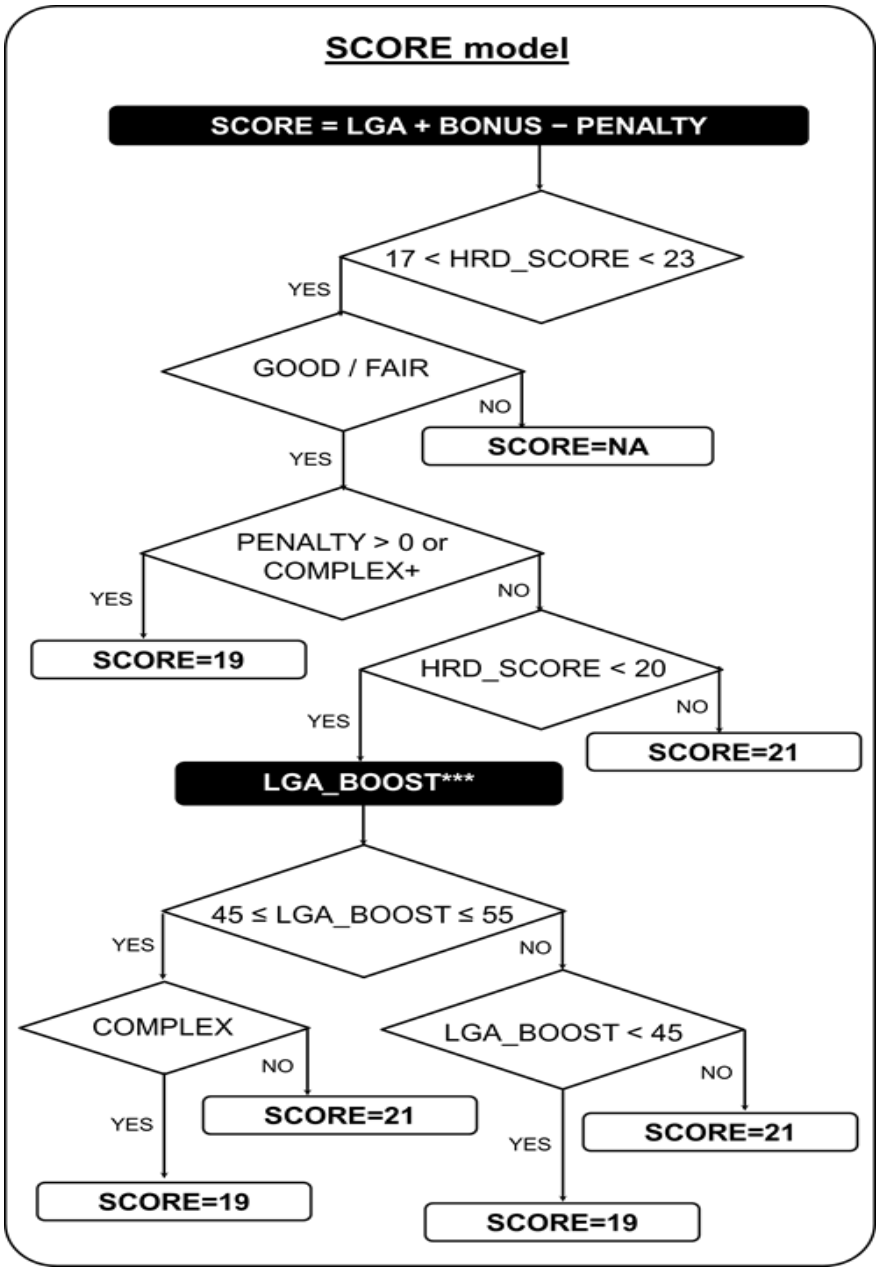
SCORE and LGA_boost in COMPLEX genome



Points are tumor genomes,
Circles indicate standard errors of LGA in 20 runs

HRD call rule: COMPLEX genomes at the borderline and
LGA_boost ≥ 55 or SCORE ≥ 20 → **HRD**

Simplified DECISION TREE



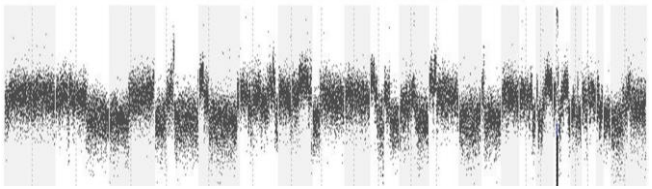
shallowHRD_v2

Workflow

FF or FFPE tumor sample

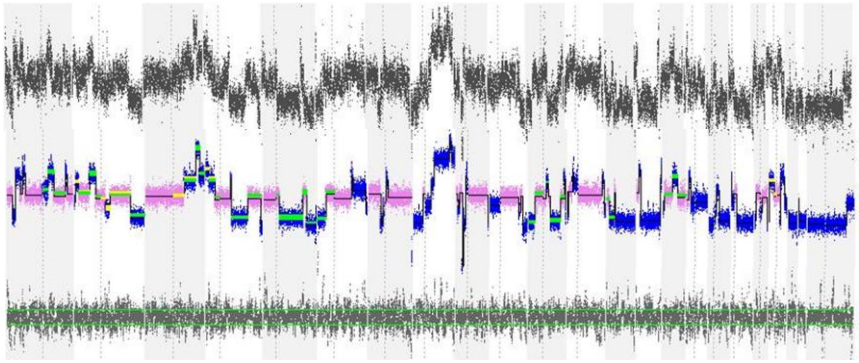
DNA extraction
with min 30% tumor cells

shallowWGS
Whole Genome Sequencing (~1X)



Genomic CNA profile
Read Depth and GC normalization
(controlFreec)

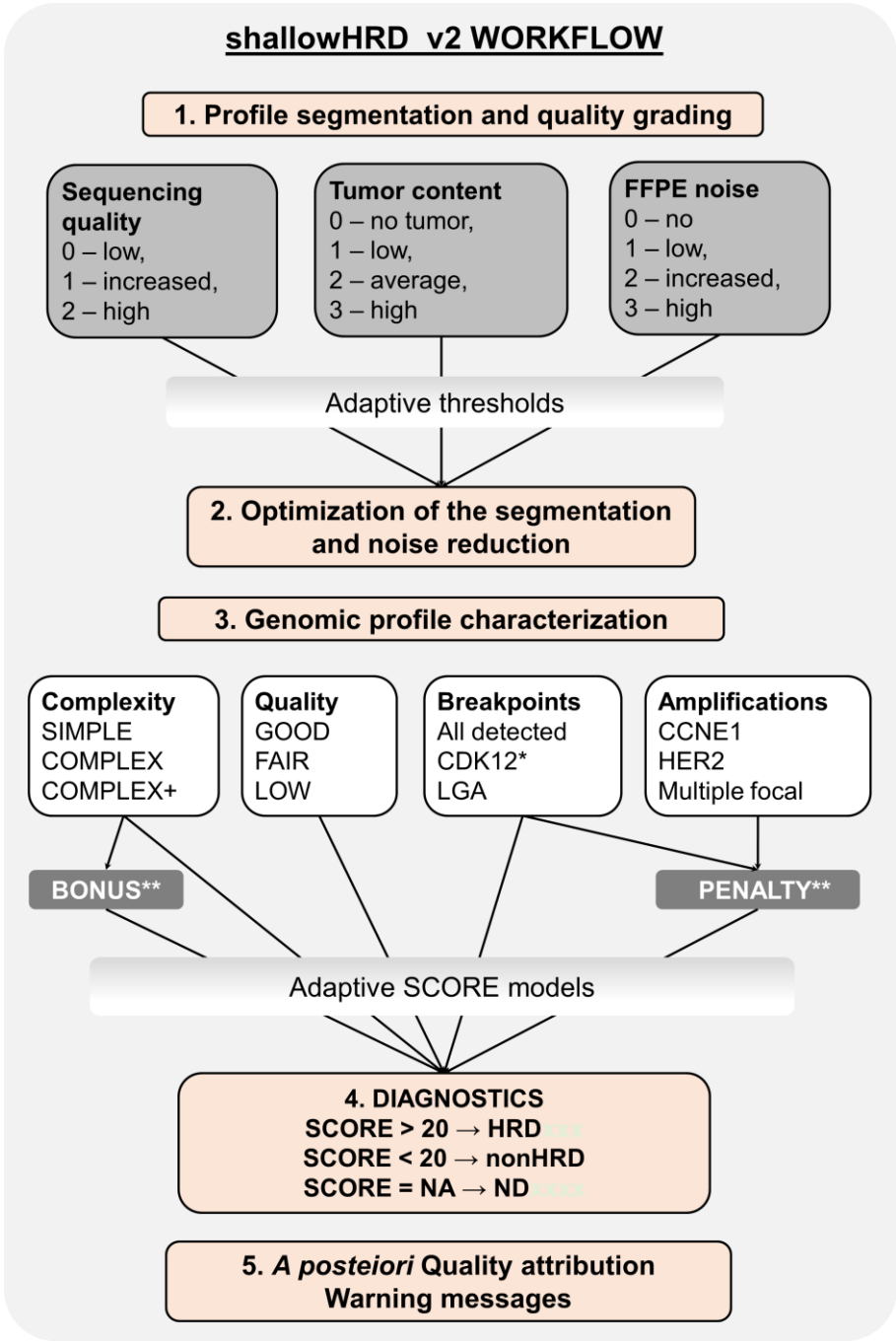
REPORT



HRD status =	HRD
SCORE =	24
CCNE1/HER2	Not altered
shallowWGS coverage	NA
Tumor content	High
Noise level	High

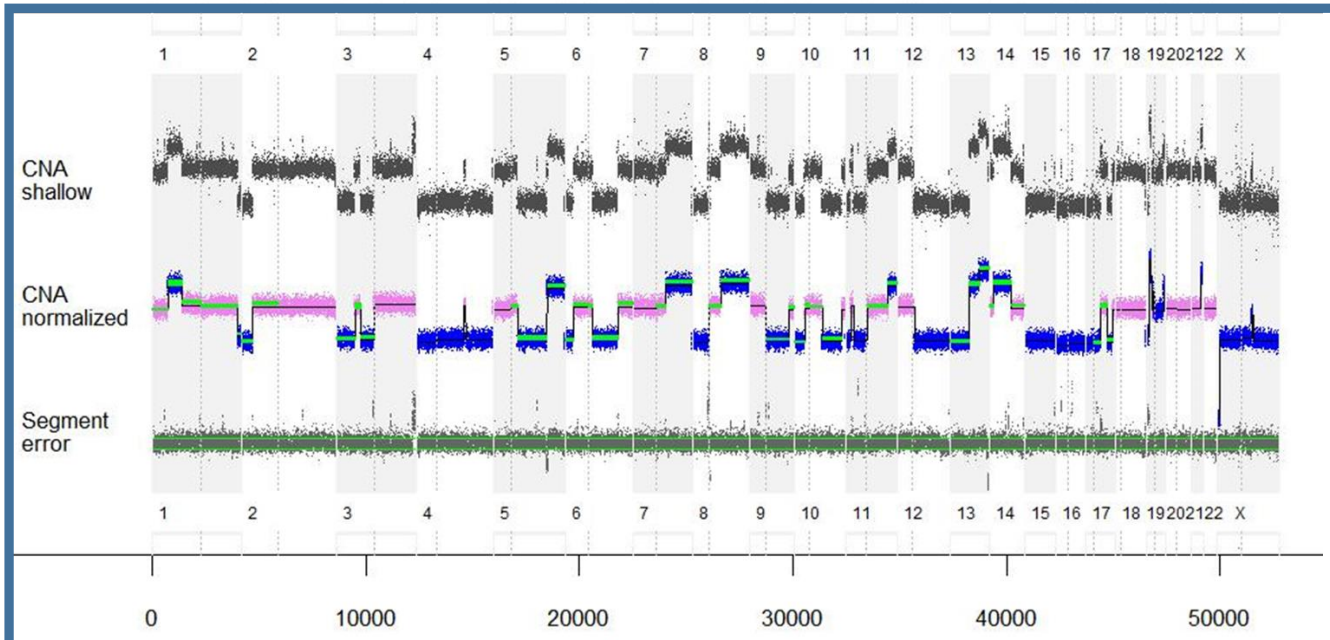
WARNING: INCREASED NOISE, INTERPRETE WITH CAUTION

Conceptual WORKFLOW:



Clinical application of shallowHRD_v2

REPORT



Summary Homologous Recombination Deficiency

HRD status =	HRD	Error rate <5% in 1000 samples
SCORE =	23	SCORE<20 -> HRD / SCORE>20 -> nonHRD
CCNE1/HER2++ =	NO	If amplified, nonHRD is highly probable
shallowWGS coverage =	2.15	< 0.5 Low coverage / > 1 Optimal coverage
Tumor content =	High	High / Average / Low / No tumor
Noise level =	Low	Low / Moderate / High / Extreme high

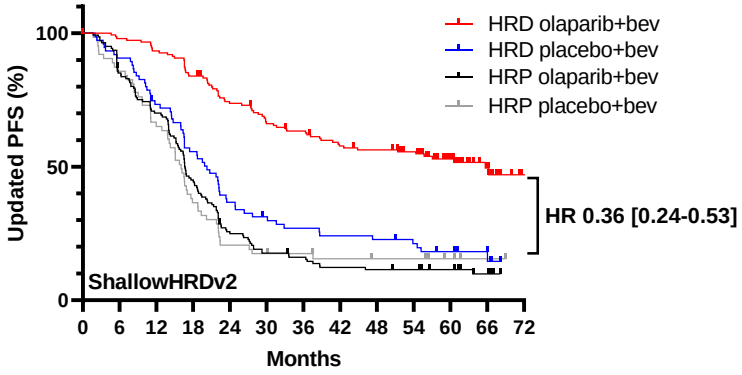
Report contains

- Profile and error representation for manual control
- Diagnostics for HRD or nonHRD
- Quality attribution
- Warnings
- Some additional features such as CCNE1 amplification, CDK12mut, etc

shallowHRD_v2 was validated in PAOLA trial and is now implemented in the hospital

Celine Callens

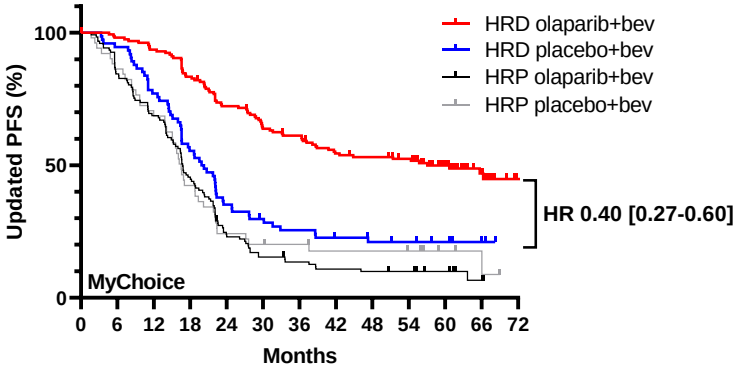
shallowHRD_v2



No. at risk:

HRD Olaparib+bev	151	148	140	126	109	97	92	83	80	73	47	23	4
HRD Placebo+bev	77	69	55	42	28	23	20	18	17	15	11	6	1
HRP Olaparib+bev	143	120	99	64	36	26	22	17	16	15	12	6	
HRP Placebo+bev	63	55	42	24	14	12	11	9	8	8	5	2	

Myriad MyChoice



No. at risk:

HRD Olaparib+bev	158	155	147	131	112	98	93	82	79	74	49	25	4
HRD Placebo+bev	76	71	58	42	27	22	19	16	14	13	11	5	1
HRP Olaparib+bev	124	103	84	56	29	20	16	13	12	11	8	3	
HRP Placebo+bev	51	45	35	22	13	11	10	8	8	7	4	3	

shallowHRD_v2: PFS is the same as Myriad MyChoice
shallowHRD_v2: ~10% less unclassified cases
shallowHRD_v2: proven patient benefit in these cases
shallowHRD_v2: 10 times less expensive!

Performance

Training set

High quality, high tumor content cases: ~10 clear mistakes for 1000 cases
Low quality or low tumor content cases:
ND cases: ~10-15%

Validation set

~3% discrepancy between Myriad MyChoice and shallowHRD_v2

Testing set

PAOLA clinical trial, when response and PFS were considered to be criteria for classification, and included mutation calls.
PAOLA data showed that manual annotation was mainly correct with only 1-2% errors in ~500 cases.

ERORS systematic

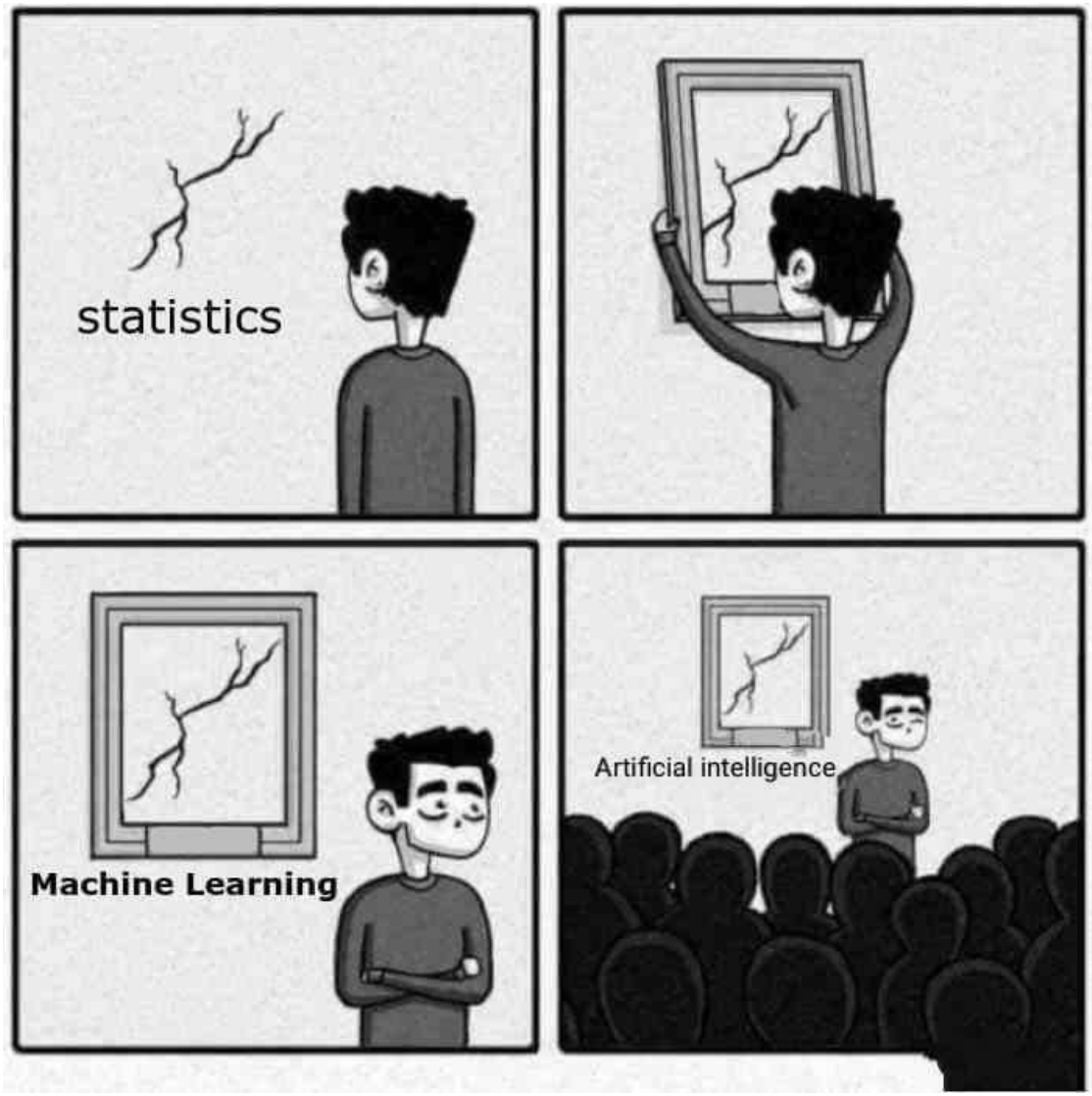
Artifacts of noise correction:

- NEOPEMBROV 2 cases from ~10 duplicates produce contradictory diagnostics
- some few more from low tumor content?

“HRD-like” cases with low Myriad score (because probably low LOH)

Few BRCA1 cell lines with true out of boundary SCOREs

Methodological conclusion



80% of cases are ~**100%** correctly classified with **1 PARAMETER LINEAR RULE**

The rest **20%**, even quasi-randomly attributed with 50% true calls, brings recognition rate to **90%**!

However, some more complex algo could increase **clinical confidence**

However, ~100 cases with borderline SCORE available to the moment are not enough for any automatic or image analysis

Commercial conclusion

There was a DREAM to make shallowHRD independent of OUR OWN PATENT ☺

However, the notion of the CNA BREAKPOINT was the key points in Curie Patent

Eventually, all who are using breakpoints (!) have to pay royalty (to us)

Image recognition is possible, but (!) to resolve borderline cases one needs much more profiles available.

There are some new solutions on the market, including AI/ML approaches. However, no one had enough samples in the training set to address borderline cases.

To the moment the legal status of shallowHRD_v2 is not clear

Scientific conclusion

1. HRD detection is quite good with shallowHRD_v2 and is in use for
    - testing patients with ovarian cancer in clinical settings
    - functional annotation of VUS in BRCA1/2 RAD51 paralogs, and other possible rare mutations
    - PDX characterization in clinical research

2. Borderline cases represent an interesting object to analyze, sensitivity to drugs, etc.

3. Interesting conclusion for data analysis: when criteria of classification at the borderline are not clear and class probability at the boundary are equal, moving the separation to either side decrease the error and increase robustness (!).

Thank you!

Marc-Henri Stern
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Victor Renault
Eleonore Frouin

Alexandre Houy
Andre Bortolini Silveira

DRUM team and U830
And all all all others!!
Now and ever before or after!

Genetics and Clinics
Ivan Bieche
Julien Masliah-Planchon
Elisabetta Maragnony
Dominique Stoppa-Lyonnet
Francois-Clement Bidard
And all others!!

DRUM team

Sequencing and
Bioinformatics platforms!