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# Global-screening array for the assessment of homologous recombination deficiency (HRD) in epithelial ovarian cancer

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# Background

In Austria ovarian cancer (OC) has incidence and mortality rates of 16 and 10.4 in 100,000, respectively[1]. The five-year overall survival is 43%[1].
Homologous recombination deficiency (HRD) is a predictive biomarker for the response of different cancer types to PARP-inhibitor therapy. About 50% of epithelial ovarian cancer (EOC)[2] are HRD positive, and, in EOC, this is also considered predictive for sensitivity to platinum-based therapies. In 40-50% of cases HRD is caused by *BRCA1/2* pathogenic variants (PVs). But, according to our current knowledge also PVs in several other genes involved in homologous recombination repair (HRR), epigenetic silencing of *BRCA1* and *RAD51C,* but probably also yet unknown mechanisms, can be responsible for HRD[2] (*Figure 1*).
Testing for all possible reasons for HRD as a biomarker for treatment response is clinically impractical. However, assessment of HRD, independently of the underlying causative mechanism can be performed by detection of genomic scars resulting from the impaired HRR.
Currently, diagnostic testing for such genomic scars is mainly provided by commercial diagnostic service companies with limited transparency of the underlying algorithms for the individual tests.
As a part of our tumor profiling platform we implemented and validated a simple and cost-effective method for HRD-assessment based on measurement of copy-number variations (CNVs) and allelic variations utilizing SNP-Array (GSA) and published algorithms for quantification of the data.

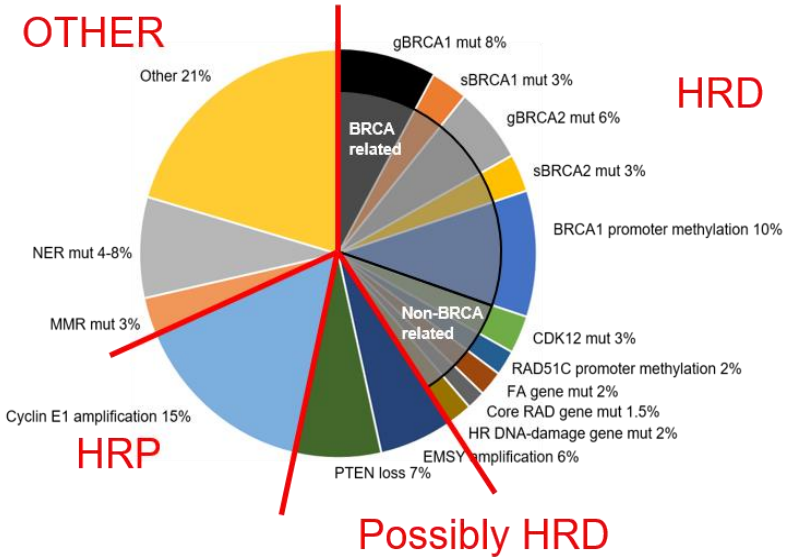



**Figure 1.** Homologous recombination deficiency (HRD) in EOC. Frequency of HRD and its causes in EOC. Fractions termed Possibly HRD, OTHER or HRP (homologous recombination proficiency) may contain HRD-positive tumors with decreasing probability. The underlying mechanisms for HRD in theses cases are not yet fully understood (modified from Konstantinopoulos et al[2]).


1 - Statistik Austria (2018); www.statistik.at
2 - Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous recombination deficiency: Exploiting the fundamental vulnerability of ovarian cancer. Cancer Discov (2015), 5 (11): 1137-54.

# Method

To determine HRD positivity we examined genome-wide copy number variation and allelic variations by genotyping 67 ovarian cancers, 25 of which contained a *BRCA1/2* PV ($BRCA_{mut}$), using the Global Screening Array (GSA-24 v3.0+Multi-Disease Content; Illumina). Data analysis was performed with Illumina GenomeStudio 1.6.3 (Genotyping Analysis Module) and NxClinical (Biodiscovery, SNP-FASST2-Segmentation Algorithmus) software. For quantification of HRD a loss of heterozygosity (LOH)-score and an Aneuploidy Normalized Telomeric Imbalance-Score were defined (see data interpretation/calculation of scores below).

**Sample** → **Data generation** → **Data analysis** → **Data interpretation/calculation of scores**

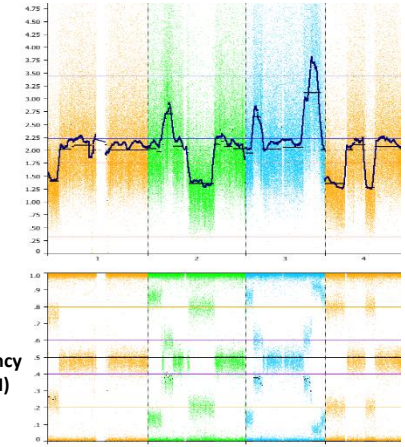
**DNA**

**Fresh frozen (>20% tumor cell content)**

**Or**

**FFPE (>50% tumor cell content)**


Illumina Global Screening Array
**>700K SNPs**




NxClinical (Biodiscovery, SNP-FASST2-Segmentation Algorithmus)

**LOH-Score[3]**

- **Percent of genomic regions meeting all of the following criteria**
  - **Spanning less than 90% of a chromosome arm (breakpoint within chromosome arm)**
  - **Copy number > 0**
  - **Minor allel = 0**
- **Limitations:**
  - **Tumors with a mostly non-diploid karyotype cannot be analysed → Minor allel = 0 criterion is not applicable**

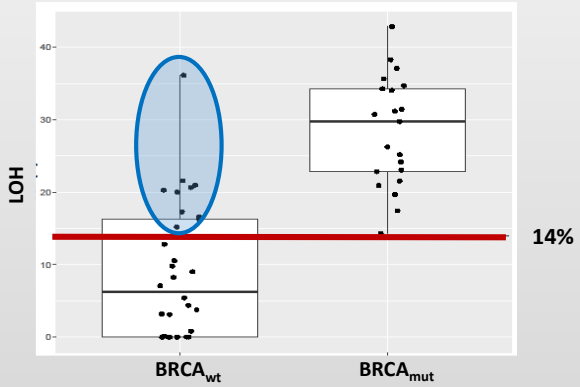
**Aneuploidy Normalized Telomeric Imbalance (ANTI)-score[4]**

- **Number of chromosomal regions meeting all of the following criteria:**
  - **Ranging from the telomere but not crossing the centromere (breakpoint within chromosome arm)**
  - **MCP-score (CN major allel/(CN major allel+CN minor allel)) > 0.7**
  - **> 12Mb**


3 - Frampton GM, Fichtenholtz A, Otto GA, Downing SR, He Jie et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biothechnol (2013), 31 (11): 1023-31.
4 - Birkbak NJ, Wang ZC, Kim JY, Eklund AC, Li Q et al. Telomeric allelic imbalance indicates defectiveDNA repair and sensitivity to DNA damaging agents. Cancer Discov (2012), 2(4): 366-75.

# Highly significant HRD assessment in validation cohort

**A** LOH-score $BRCA_{wt}$ vs $BRCA_{mut}$

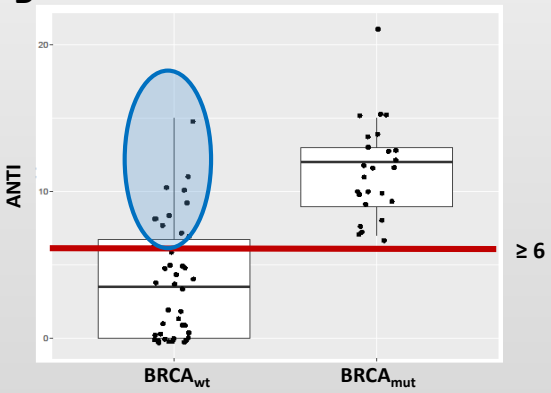



| | $BRCA_{wt}$ | $BRCA_{mut}$ | Total |
|---|---|---|---|
| n* | 32 (42) | 21 (25) | 53 (67) |
| Median | 6.3 | 29.8 | 17.3 |
| Mean (SD) | 8.9 (9.3) | 28.3 (7.6) | 16.6 (12.9) |
| Range | 0.0 - 36.1 | **14.3** - 42.9 | 0.0 - 42.9 |

**Wilcoxon rank-sum test: p<0,001**

*Assessed samples (total samples); not all samples are eligible for LOH-score assessement.

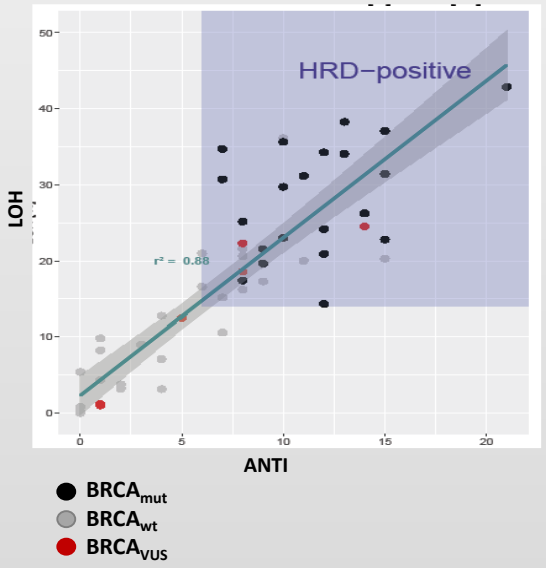
**B** ANTI-score $BRCA_{wt}$ vs $BRCA_{mut}$




| BRCA-Mut | $BRCA_{wt}$ | $BRCA_{mut}$ | Total |
|---|---|---|---|
| n | 42 | 25 | 67 |
| Median | 3.5 | 12.0 | 7.0 |
| Mean (SD) | 3.8 (3.9) | 11.5 (3.3) | 6.7 (5.2) |
| Range | 0.0 - 15.0 | **7.0** - 21.0 | 0.0 - 21.0 |

**Wilcoxon rank-sum test: p<0,001**

**C** Correlation of LOH- and ANTI-score




The group of samples with *BRCA1/2*-PV ($BRCA_{mut}$) had significantly higher median scores than *BRCA1/2*-wildtype samples ($BRCA_{wt}$): LOH-score: 29.8 vs. 6.3 (**A**); ANTI-score: 12 vs. 3.5 (**B**). LOH-score and ANTI-scores were concordant ($R^2$= 0.88; **C**). Based on the lowest scores determined in the $BRCA_{mut}$ samples, we defined the threshold for HRD-positivity as LOH-score ≥14 (**A**) and/or ANTI-score ≥6 (**B**). Using these threshold scores, 10/32 and 12/42 of $BRCA_{wt}$ samples had LOH- and ANTI-scores, respectively, above the thresholds (highlighted blue in **A** and **B**) indicating HRD due to underlying mechanisms other than *BRCA1/2*-PVs.

# Conclusions and Outlook

**Conclusion:**

- Determination of HRD in routine diagnostics with a low cost generic SNP-array
- Two scores improve reliability of results and allow to compensate for limitations of the individual scores (e.g. non-diploid karyotype in case of LOH-score).
- This method is an integral part of our tumor profiling platform for gynecological tumors (FFPE and fresh frozen material).

**Work in progress:**

- Testing HRD positives from the $BRCA_{wt}$ validation cohort for other (epi-)genetic aberrations explaining their HRD positivity.
- Proficiency testing by sample exchange with other providers of HRD assessment. Preliminary data show high concordance of the results with a commercial laboratory.
- Validation of the method for other tumor entities (e.g. prostate cancer, pancreatic cancer)

**Future plans:**

- Evaluation of correlation of treatment response to PARP-inhibitor- and/or platinum-based therapy and increasing HRD scores in patients with HRD-positive tumors.
- Integration of tumor HRD score analysis into a multi-tier classification scheme of variants of unknown significance (VUS, *Figure 4*) .

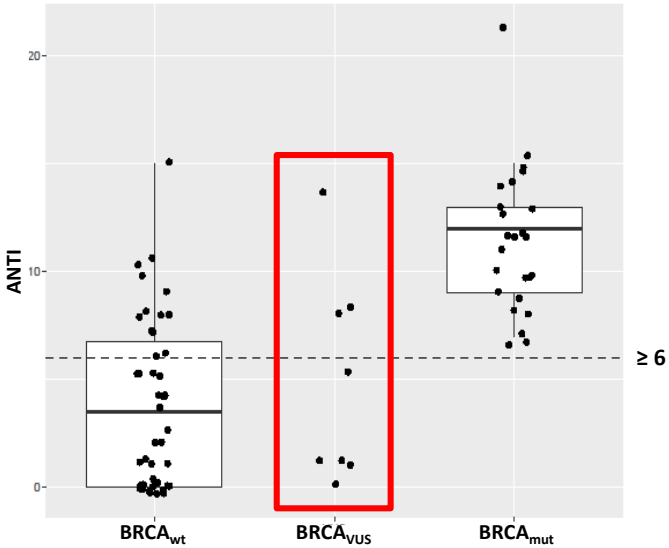



**Figure 4:** Results of HRD testing results of eight EOC-samples with BRCA-VUS ($BRCA_{VUS}$; higlighted by red rectangle) in comparison to our validation cohort of $BRCA_{wt}$ and $BRCA_{mut}$ samples.