An Analysis of 13 Independently Performed Assays to Measure Homologous Recombination Deficiency Using 90 Freshly Extracted High Grade Serous Ovarian Tumors: Findings from the Friends of Cancer Research HRD Harmonization Project

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Introduction

Homologous recombination deficiency (HRD) assays determine eligibility for treatment with PARP inhibitors and may have use for other DNA repair targeting drugs. The assays measure several factors to define homologous recombination (HR) status including causes (i.e., inactivation in HR repair (HRR) pathway genes) and consequences (i.e., genomic scarring) of HRD. Variability in determining HR status across HRD assays has not been investigated thoroughly, and an empirical assessment of assay variability may support broader adoption of HRD and strengthen clinical interpretation of test results.

Materials & Methods

HRD Assays

Commercial and academic HRD assay developers were invited to participate in the project, resulting in 16 organizations representing 18 HRD assays. Factors measured to determine HR status (i.e., gLOH Inclusion, TAI Inclusion, LST inclusion, mutations in non-BRCA1/2 HRR pathway genes) were provided by the test developers. For each sample, developers provided HRD status, score, and BRCA1/2 status. There are research use only (RUO) assays and laboratory developed tests (LDTs) included in the analyses.

In Silico Samples

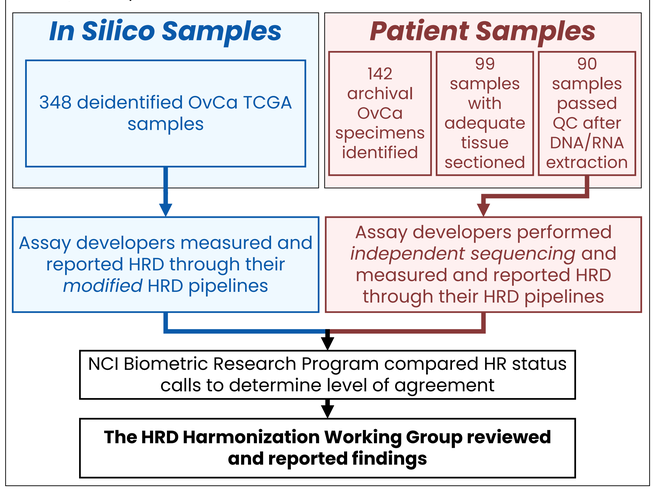
A subset of assay developers (n=11) received de-identified segmented files,[i] MAF files,[ii] and BRCA1/2 germline mutation files for 348 TCGA ovarian cancer samples.[iii] Assay developers ran TCGA samples through their modified HRD pipeline to measure and report HR status and the contributing factor(s) for each sample. BRCA1/2 mutated samples were defined as samples included in the germline mutation file[iii] and samples in which any group identified a somatic *BRCA1* or *BRCA2* alteration (n=83).

Patient Samples

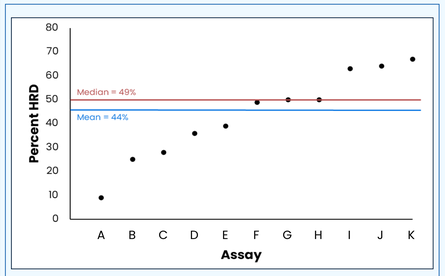
Archival specimens (n=142) from patients with stage III-IV high grade serous ovarian cancer diagnosed between 2011 and 2022 were identified in a biorepository at the University of Alabama at Birmingham (UAB). UAB sectioned FFPE tumor from debulking surgery for the 99 samples with adequate tissue and Molecular Characterization Laboratory (MoCha) at the NCI Frederick National Laboratory performed DNA and RNA extraction. MoCha shipped identical aliquots of DNA and/or RNA from the 90 samples that passed QC for independent sequencing and HRD measurement by 13 assays. *BRCA1* and *BRCA2* alterations were defined by clinical data from UAB, which included germline and somatic alterations.

Statistical Analysis

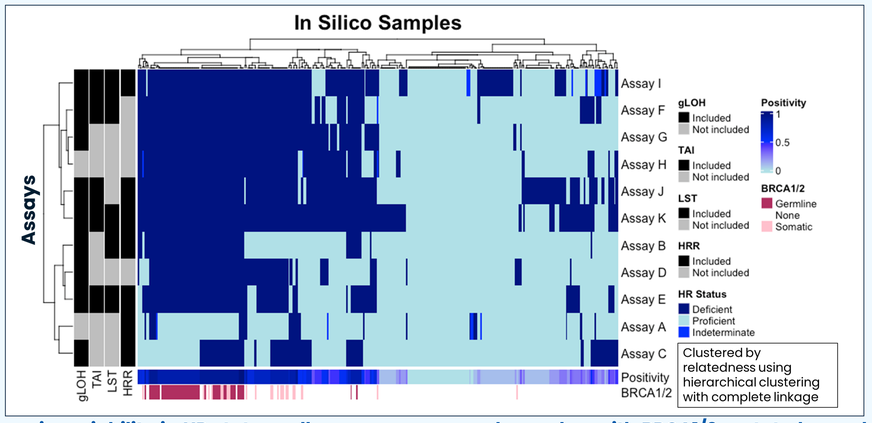
Statisticians from the NCI Biometric Research Program performed pairwise comparisons of assays' HR status calls to determine the level of agreement and considered specific factors measured by each assay to identify potential sources of variation for each dataset (In Silico Samples and Patient Samples were analyzed separately). Additionally, they analyzed HR status agreement for BRCA1/2 mutated versus wild type BRCA1/2 samples.



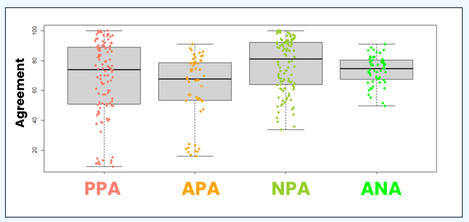
In Silico Sample Results



The range of percent HRD positivity is 9-67% with a median of 49% and a mean of 44%. Assay developers (n=11) ran ovarian cancer TCGA samples (n=348) through their HRD pipelines and reported whether each sample was HRD or not. The percent of samples that were HRD out of all the samples was reported as the percent HRD for each assay. The assays are ordered by percent HRD here and throughout the analysis.



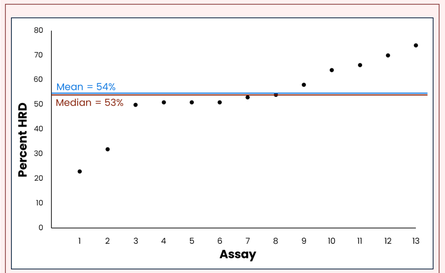
There is variability in HR status calls across assays and samples, with BRCA1/2 mutated samples more uniformly called HRD. The tile plot depicts HRD calls by all assays (n=11) for all in silico samples (n=348). Assays and samples are also clustered by relatedness using hierarchical clustering with complete linkage. Assay factors are depicted as yes/ no based on whether the factor to determine HR status was included in the assay algorithm (HRR=alterations in non-BRCA1/2 HRR pathway genes as defined by the developer).



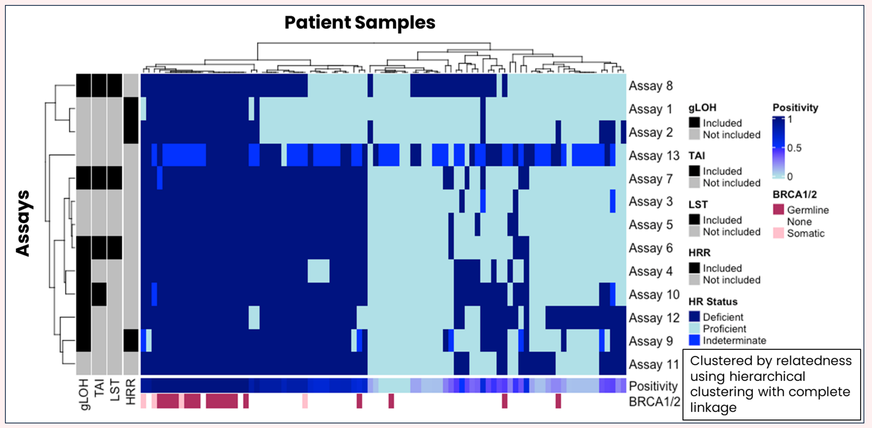
	Min.	1Q	Med.	Mean	3Q	Max.
PPA	9	51	**74**	68	89	100
APA	16	53	**68**	62	78	91
NPA	34	64	**81**	77	92	100
ANA	50	67	**75**	74	80	91

Positive/negative agreement varied across assays, with modest to high levels of agreement. Percent positive agreement (PPA), negative positive agreement (NPA), average positive percent agreement (APA), and average negative percent agreement (ANA) were computed for all possible pairings of samples (n=348) and assays (n=11).

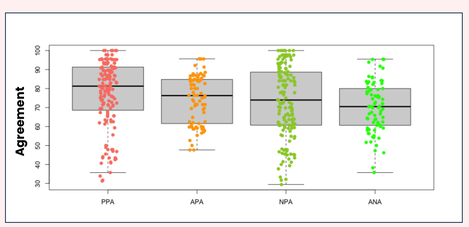
Patient Sample Results



The range of percent HRD positivity is 23-74% with a median of 53% and a mean of 54%. Assay developers (n=13) ran ovarian cancer patient samples (n=90) through their HRD pipelines and reported whether each sample was HRD or not. The percent of samples that were HRD out of all the samples was reported as the percent HRD for each assay. The assays are ordered by percent HRD here and throughout the analysis.



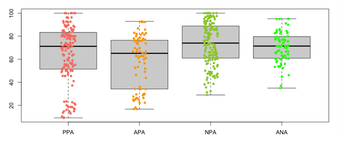
There is similar variability in HR status calls across assays and samples, with BRCA1/2 mutated samples more uniformly called HRD. The tile plot depicts HRD calls by all assays (n=13) for all samples (n=90). Assays and samples are also clustered by relatedness using hierarchical clustering with complete linkage. Assay factors are depicted as yes/ no based on whether the factor to determine HR status was included in the assay algorithm.



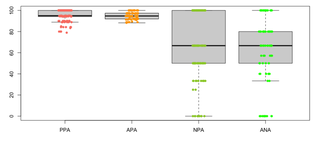
	Min.	1Q	Med.	Mean	3Q	Max.
PPA	31	69	**81**	77	91	100
APA	48	62	**76**	74	85	96
NPA	29	61	**74**	73	89	100
ANA	36	61	**70**	70	80	95

Positive/negative agreement varied across assays for patient samples, with modest to high levels of agreement. PPA, NPA, APA, and ANA were computed for all possible pairings of samples (n=90) and assays (n=13).

Agreement Among Samples with WT *BRCA1* and *BRCA2*



Agreement Among Samples with Altered *BRCA1* or *BRCA2*



PPA is higher in BRCA1/2 altered samples, NPA is lower. PPA, NPA, APA, and ANA were computed for all possible pairings of samples with WT *BRCA1* and *BRCA2* (n=68) and for samples with altered *BRCA1* and/or *BRCA2* (n=22) across all assays (n=13). Similar results were seen for the In Silico Analysis (data not shown).

Spearman Correlation Summary Statistics

		Min.	Med.	Mean	Max.
HRD Score	ALL	0.19	**0.72**	0.69	0.97
	Non-BRCA1/2	0.13	**0.68**	0.67	0.97
%gLOH	ALL	0.19	**0.57**	0.61	1.00
	Non-BRCA1/2	0.10	**0.47**	0.54	1.00

Correlations among continuous HR scores varied substantially across assays. Spearman correlation coefficients were calculated between each pair of assays that provided continuous HRD scores (n=10) and for each pair of assays that provided continuous %gLOH scores (n=5). The Spearman correlation is based on ranks (assays have different scales). Each assay developer had their own copy number modeling and segmentation, which may account for low correlations.

Conclusions

This unique partnership allowed us to further understand similarities and differences among HRD assays.

- The median HRD positivity rate of 49% in the In Silico Analysis and 53% in the Patient Sample Analysis is consistent with prior publications.
- For both analyses, the inter-assay agreement on HR status calls was variable. In the In Silico Analysis, it does not appear to be strongly driven by which factors were included in the algorithms, whereas results for some samples in the Patient Sample Analysis may be driven by the inclusion of "consequences." Future research should consider the role of causes vs. consequences in HRD score determination.
- Median PPA among samples with altered *BRCA1* and *BRCA2* is greater than those with WT *BRCA1* and *BRCA2* while median NPA is lower demonstrating the influence of *BRCA1* and *BRCA2* on HRD calls.

Understanding the agreement among assays will inform assay interpretation and improve alignment of HRD scores to help patients and providers make appropriate treatment decisions.

Next Steps

- Perform additional analyses that examine the impact of clinical factors (e.g., platinum status, race), sample factors (e.g., DNA quality, tumor content), and alterations in HRR pathway genes (e.g., *RAD51C*, *PALB2*) on HRD call concordance.
- Report findings and provide recommendations for future use of HRD assays – *Friends* will host a public meeting on February 1, 2024.

References

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ii. Data Types Collected by TCGA https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/types Whole Genome MAF Files
iii. Maxwell, K. N., et al. (2017). BRCA locus-specific loss of heterozygosity in germline BRCA1 and BRCA2 carriers. Nature Communications 2017 8:1, 8(1), 1–11. Supplementary Data File 1