Assessing Variability Across HRD Assays: Findings from the *Friends'* HRD Harmonization Project

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Introduction

Homologous recombination deficiency (HRD) assays determine eligibility for treatment with PARP inhibitors and potentially 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. Methodological variability 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

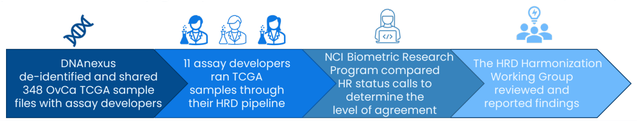
Assay Factors

We surveyed HRD assay developers (n=20) about factors their assays measure to determine HR status.

Surveyed Assay Factors
HRD Score
gLOH Inclusion
gLOH Cutoff
BRCA1/2 Inactivation
TAI Inclusion
LST Inclusion
Methylation in non-BRCA HRR Pathway Genes
Mutations in non-BRCA HRR Pathway Genes
Sig 3 Inclusion

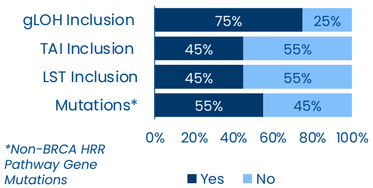
In Silico Analysis

A subset of assay developers (n=11) received de-identified segmented files,[i] MAF files,[ii] and BRCA 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. 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. Additionally, they analyzed HR status agreement for BRCA1/2 mutated versus wild type BRCA1/2 samples. BRCA1/2 mutated samples were defined as samples included in the germline mutation file[iii] and samples in which any group identified a BRCA1 or BRCA2 alteration (n=83).



Results

Assay Factors



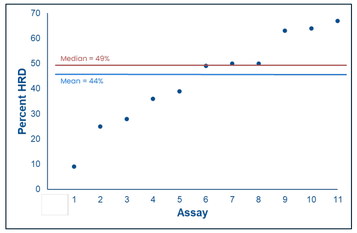
**Non-BRCA HRR Pathway Gene Mutations*

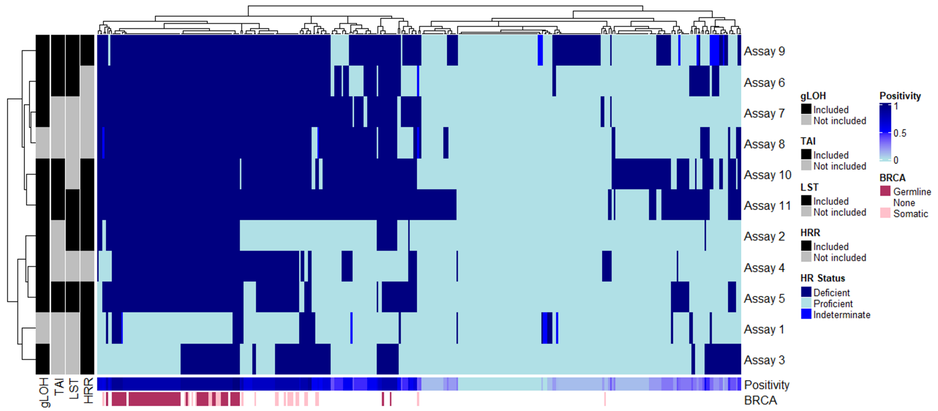
Assays vary in which factors are included in the HRD analysis pipeline. Assay developers (n=20) were surveyed to determine factors included in their algorithms to determine HRD. All groups measure *BRCA1* and *BRCA2* mutations (graph depicts those who measure genes other than *BRCA1* and *BRCA2*). None of the groups reported measuring methylation in HRR pathway genes. Assays included in the *in silico* analysis had a similar trend for assay factor inclusion.

Results

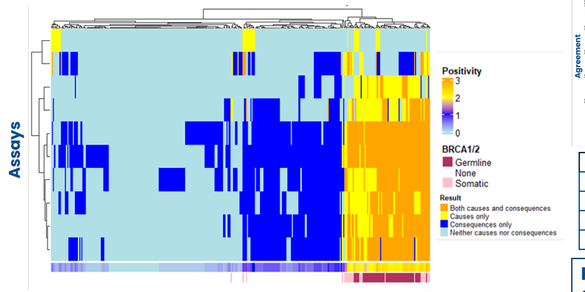
In Silico Analysis

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.

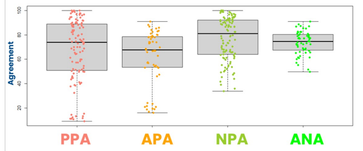




There is variability in HR status calls across assays and samples, with BRCA1/2 mutated samples more uniformly called HRD. The tile plot depicts HRD calls by all assays (n=11) for all samples (n=348). Assays and samples are also clustered by relatedness using hierarchical clustering with complete linkage.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



There is moderate agreement between assays for both causes and consequences, but concordance is higher for causes than for consequences. For each sample (n=348), assays (n=9) provided whether causes or consequences determined the HR status call and results were combined into a tile plot. Assays and samples are both clustered by relatedness using hierarchical clustering with complete linkage.

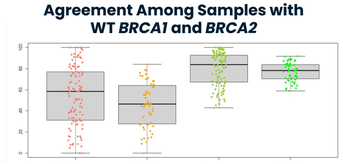


	Min.	1Q	Med.	Mean	3Q	Max.
PPA	9	51	**74**	68	89	100
APA	16	53	**68**	62	78	91
NPA	34	64	**81**	77	92	100
ANA	50	67	**75**	74	80	91

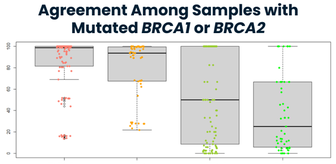
Positive/negative agreement varied across assays, with modest to high levels of agreement. Percent positive agreement (PPA), negative positive agreement (NPA), average positive percent agreement (APA), and average negative percent agreement (ANA) were computed for all possible pairings of samples (n=348)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

Results

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



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



PPA is higher when only samples with BRCA1/2 mutations are considered, NPA is lower. PPA, NPA, APA, and ANA were computed for all possible pairings of samples with WT *BRCA1* and *BRCA 2* (n=265) and for samples with altered *BRCA1* and/or *BRCA2* (n=83) across all assays (n=11).

CS Value	Assay Outcome	Result Options
0	Opposite	+/- or -/+
1	Same	+/+, -/-, or in/in

Y	CS	SE	95% CI	
HR Status	0.705	0.009	0.687	0.724
Causes	0.872	0.008	0.856	0.888
Consequences	0.680	0.010	0.661	0.700

Concordance for HR status is moderate with high concordance for causes and lower concordance for consequences. For each comparison, a concordance score (CS) was calculated using a CS Value = 0 if the assays have the opposite outcome and a CS Value = 1 if the assays have the same outcome. To determine the overall concordance, the score was averaged over samples and assays. (CS Value = undefined if "+/in" or "-/in" which was 1% for HR status, 18% for Causes, and 0% for Consequences.)

HRD Score
Spearman Correlation summary statistics

	Min.	Med.	Mean	Max.
ALL	0.20	**0.66**	0.62	0.93
Non BRCA	0.17	**0.64**	0.60	0.91

%gLOH
Spearman Correlation summary statistics

	Min.	Med.	Mean	Max.
ALL	0.52	**0.70**	0.74	1.00
Non BRCA	0.50	**0.66**	0.73	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=8) and for each pair of assays that provided continuous %gLOH scores (n=6). The Spearman correlation is based on ranks (assays have different scales). Since identical data inputs were used, low correlations are not explained by differences in copy number modeling or segmentation.

Conclusions

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

- While gLOH is presently the most used factor in HRD analysis pipelines (75%), most assays used multiple factors.
- The median HRD positivity rate of 49% is consistent with prior publications. The positivity rate varied widely across assays (9 to 67%).
- The inter-assay agreement on HR status calls was variable but was not governed by which factors were included in the HRD scores, thus, emphasizing the importance of developing best practices.
- There was more variability in approaches for measuring consequences versus causes and concordance for causes (0.87) was greater than concordance for consequences (0.68).

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

An analysis of freshly extracted formalin-fixed paraffin-embedded human archival ovarian tumor samples is planned for early 2023, which will provide additional context for interpreting the findings from the *in silico* dataset.

References: i. PanCan Atlas https://gdc.cancer.gov/about-data/publications/pancanatlas - ABSOLUTE-annotated seg file - TCGA_mastercalls.abs_segtabs.fixed.txt 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 81, 8(1), 1–11. Supplementary Data File 1