Introduction
Homologous recombination deficiency (HRD) assays determine eligibility for treatment with PARP inhibitors and may have use for upfront DNA repair testing. The assays measure several factors to define homologous recombination (HR) status including causes (i.e., inactivation in HR repair (HRR) pathway genes) and consequences (e.g., genomic scoring) 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 13 groups representing 13 different HRD assays. Factors measured to determine HR status (i.e., gLOH inclusion, TAI inclusion, LST inclusion, mutations in non-BRCA1/2 HR 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, MAF files, and BRCA1/2 germline mutation files for 348 TCGA ovarian cancer samples. Assay developers ran TCGA samples through their modified pipelines to measure HR status and the contributing factors for each sample. BRCA1/2 mutated samples were defined as samples included in the germline mutation file and samples for which any group identified a somatic BRCA1 or BRCA2 alteration.

**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 biopspecimen 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 (MCf) at the NCI Frederick National Laboratory performed DNA extraction. Mcf-TCGA samples were 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 Friends of Cancer Research Biometric Research Program performed pairwise comparisons of assay 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 Results**
  - The range of percent HRD positivity is 0.57% to 53.00%
  - There is variability in HR status calls across assays and samples, with BRCA1/2 mutated samples more uniformly called HRD.

- **Patient Results**
  - The range of percent HRD positivity is 23.74% to 53.00%
  - There is similar variability in HR status calls across assays and samples, with BRCA1/2 mutated samples more uniformly called HRD.

Conclusions
This unique partnership allowed us to further understand similarities and differences among HRD assays. The median HRD positivity rate of 46% in the In Silico Samples and 53% in the Patient Sample Analysis is consistent with previous 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 algorithm, 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.

- **Assay developers performed independent sequencing and reported HRD through their pipelines**
- **NCI Biometric Research Program compared HR status calls to determine level of agreement**
- **The HRD Harmonization Working group reviewed and reported findings**

**Next Steps**
- Perform additional analyses that examine the impact of clinical factors (e.g., stage, gene status, race, sample factors, e.g., RNA 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.