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 deficiency (HRD) 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 HRD status.

In Silico Analysis
A subset of assay developers (n=11) received de-identified segmented files/MAF files2 and BRCA germline mutation files for 348 TCGA ovarian cancer samples. Assay developers ran TCGA samples through their modified HRD pipeline to measure HR status and the contributing factors(s) for each sample. Statistics from the NCI Biometric Research Program performed pairwise comparisons of HRD status calls to test the level of agreement and considered specific factors measured by each assay to identify potential sources of variation. Additionally, they analyzed HR status agreements for BRCA1/2 mutated versus wild type BRCA1/2 samples. BRCA1/2 mutated samples were defined as samples included in the germline mutation file3 and samples in any group that included a BRCA1 or BRCA2 alteration (n=83).

Results

Assay Factors

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). Assay 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.

Conclusions

This unique partnership allows 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 do not appear to be strongly driven by which factors were included. HRD positivity is strongly driven by 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 improvement of different HRD scores to help patients and providers make appropriate treatment decisions.

An analysis of freely extracted formatted fixed-paradigm bi-amplified human archival tumor samples is planned in order to provide additional context for interpreting the findings from the in silico analysis.

References: