Assessment of Homologous Recombination Deficiency and *BRCA* Status in Ovarian Cancer: Analytical Performance and Relevance of a Decentralized NGS Assay for Comprehensive Genomic Profiling

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Background

- **Homologous Recombination Deficiency (HRD)** is a complex biomarker with predictive value in ovarian cancer.
- Understanding **both the causes of HRD**, such as pathogenic alterations in homologous recombination repair (HRR) genes, **and its consequences like genomic instability (GI)**, is crucial for exploring therapeutic strategies, including the potential use of poly (ADP-ribose) polymerase inhibitors (PARPi).
- This study evaluates the **analytical performance and clinical research relevance of the Oncomine™ Comprehensive Assay Plus (OCA Plus)**, a distributable next-generation sequencing (NGS) assay that offers in a single workflow comprehensive genomic profiling (CGP), including HRD evaluation.

Methods

- **OCA Plus allows for CGP of solid tumors** using Ion Ampliseq™ technology, automated templating on Ion Chef™ system and sequencing on the Ion GeneStudio™ S5 platform. The assay covers **500+ genes relevant to precision oncology research including *BRCA1/2* and 45 other genes in HRR pathway**.
- **GI status was determined using Genomic Instability Metric (GIM)**, a novel metric to quantify genomic scars/instability associated with HRD by summarizing different unbalanced copy number events across the autosomes using genomic segmentation and combined with *BRCA1/2* mutational status to determine overall HRD status (Figure 1).
- A series of **125 ovarian cancer research samples from the PAOLA-1 trial**,[1,2] part of the ARCAGY biorepository were evaluated using OCA Plus and compared with the reference method for analytical performance. Progression-free survival (PFS) was assessed for clinical research relevance.

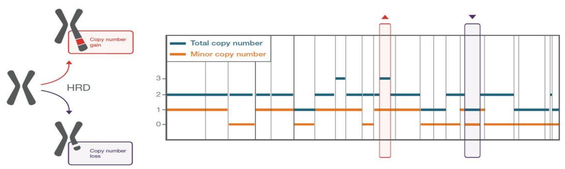


Figure 1. Examples of unbalanced CN gain and loss events that are summarized in GIM.

Key Takeaways

1. **OCA Plus is a distributable NGS assay that allows CGP for solid tumors including HRD evaluation with only 20 ng DNA input and very high sequencing success rate.**
2. **GI is characterized using a proprietary algorithm that generates a quantitative score, GIM, summarizing different unbalanced copy number events across the autosomes.**
3. **GIM status is combined with *BRCA1/2* mutational status to provide overall HRD status that has high overall percent agreement of 91% with the reference method.**
4. **PFS analysis demonstrated clinical research relevance for HRD assessment using OCA Plus with a significantly improved hazard ratio for the HRD positive cases (HR: 0.37, p < 0.005) compared to the HRD negative cases (HR: 0.91, p=0.78).**

Results

Analytical Performance

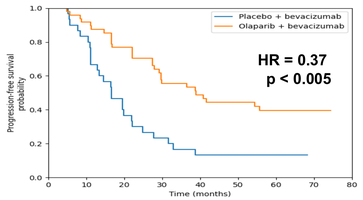
The **success rate for DNA sequencing was 100%**, starting from a **minimal sample input of 20 ng**. The OCA Plus panel provided a detailed genomic profile in a single workflow, achieving high success rates (100%) across all biomarkers tested, including single nucleotide variants, insertions and/or deletions and HRD. **High agreement was observed in *BRCA1/2* mutational status, GI status and overall HRD status** with the reference method as shown in Table 1 below.

Table 1. Analytical performance of HRD assessment using OCA Plus compared to the reference method on PAOLA-1 sub-cohort of 125 ovarian cancer samples.

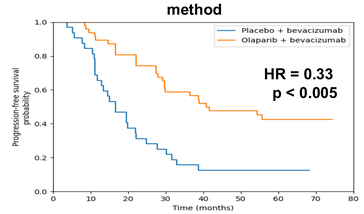
	***BRCA1/2* mutational status** (n = 125)	**GIM status** (n = 125)	**HRD status (*BRCA1/2* + GIM)** (n = 125)
Positive Percent Agreement	95.5	78.4	92.4
Negative Percent Agreement	100	88.4	89.1
Overall Percent Agreement	98.4	86.4	91.2

Retrospective Study Results

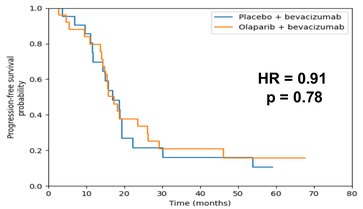
PFS in subjects with HRD positive status from OCA Plus



PFS in subjects with HRD positive status from reference method



PFS in subjects with HRD negative status from OCA Plus



PFS in subjects with HRD negative status from reference method

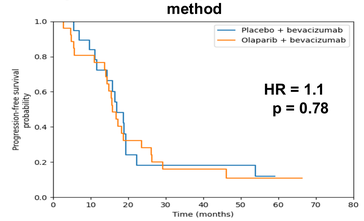


Figure 2. PFS for subjects by HRD status from OCA Plus (left panel) and reference method (right panel).

PFS for subjects taking olaparib + bevacizumab or placebo + bevacizumab was assessed and analyzed for subjects **stratified according to overall HRD status from OCA Plus** and reference method respectively (Figure 2). The **stratification with only *BRCA1/2* mutation status or only GI status from HRD assessment using OCA Plus also demonstrates significantly better hazard ratio** by itself as shown in Table 2.

Table 2. Clinical research relevance using PFS in PAOLA-1 sub-cohort of 125 ovarian cancer research samples using OCA Plus.

TREATMENT ARM	***BRCA1/2* mutational status** Hazard ratio (95% CI) p-value	**GIM status** Hazard ratio (95% CI) p-value	**HRD status (*BRCA1/2* + GIM)** Hazard ratio (95% CI) p-value
Olaparib + bevacizumab	0.23 (0.11 – 0.52) < 0.005	0.41 (0.23 – 0.73) < 0.005	0.37 (0.22 – 0.65) < 0.005
Placebo + bevacizumab	0.77 (0.47 – 1.25) 0.29	0.66 (0.36 – 1.21) 0.18	0.91 (0.48 – 1.74) 0.78

References

1. PAOLA-1 ClinicalTrials.gov number, NCT02477644. 2. Ray-Coquard I, Pautier P, Pignata S, et al. *N Engl J Med* 2019;381(25):2416−2428.

Acknowledgments

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