Assessment of Homologous Recombination Deficiency in Ovarian Cancer

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SUMMARY

Accurately assessing homologous recombination deficiency (HRD) to use as a predictive biomarker is an area of intense research in ovarian cancer. Validated assays have demonstrated utility in determining maintenance therapy following platinum sensitive chemotherapy. Novel functional assays promise the potential to reflect HRD in real time and predict response to PARP inhibitors.

See related articles by Pikkusaari et al., p. 3110 and Blanc-Durand et al., p. 3124

In this issue of *Clinical Cancer Research*, Pikkusaari and colleagues (1) and Blanc-Durand and colleagues (2) describe two novel predictive methods reflecting homologous recombination deficiency (HRD) function, using *RAD51* quantitative immunohistochemistry (qIHC) and *BRCA1* promoter methylation (BRCA1meth). Homologous recombination repair (HRR) is the most prevalent and precise pathway for the repair of double stranded DNA breaks and involves multiple genes acting in synergy to maintain and restore genomic integrity. Alterations influencing repair function in this pathway (**Fig. 1**) can occur due to germline or somatic mutations, epigenetic silencing, or aberrations in the other regulating genes, and lead to HRD (3). Germline *BRCA1/BRCA2* mutations are observed in 13% to 17% of patients with high-grade serous ovarian cancer, and additional 6% to 8% patients have somatic *BRCA1/BRCA2* mutations. In addition, HRR may be impaired due to promoter methylation, mutations in other HRR pathway–related genes, and additional genetic anomalies (4). HRD has been shown to be prognostic and validated as a predictive biomarker. Current validated HRD assays reflect genetic alterations and structural chromosomal abnormalities or genomic scars, loss of heterozygosity (LOH), large-scale transitions (LST), and telomeric allelic imbalances (TAI). There are two FDA-approved genomic scar assays myChoice HRD (Myriad Genetics) and FoundationOne CDx assay (Foundation Medicine) to detect HRD status. myChoice HRD quantifies LOH, LST, and TAI by estimating somatic copy-number variations (CNV) in single nucleotide polymorphism (SNP) and provides a consolidated genomic instability score (GIS). FoundationOne CDx assay uses hybrid capture-based next-generation sequencing (NGS) to detect SNP, insertions/deletions, CNV, and genomic alterations in 324 cancer-related genes (5, 6). They are widely used in clinical practice and through integration as companion diagnostics, have established a high predictive value for PARP inhibitors (PARPi) response in patients with advanced high-grade ovarian cancer responding to platinum-based chemotherapy.

The myChoice HRD assay was used to estimate HRD in the PRIMA trial evaluating niraparib maintenance in patients with newly diagnosed advanced high-grade ovarian cancer who responded to platinum-based chemotherapy. The median progression-free survival (PFS) was significantly improved with niraparib in BRCA-mutated [22.1 months vs. 10.9 months; hazard ratio (HR), 0.40; confidence interval (CI), 0.27–0.62], HRD-positive (21.9 months vs. 10.4 months; HR, 0.43; CI, 0.31–0.59), though more modest benefit was still seen in HRD-negative (8.1 months vs. 5.4 months; HR, 0.68; CI, 0.49–0.94) populations (7). In the ATHENA-MONO trial, FoundationOne CDx was used for HRD estimation to evaluate rucaparib maintenance. Median PFS was improved with rucaparib in BRCA-mutated (not reached vs. 14.7 months; HR, 0.40; CI, 0.21–0.75), HRD-positive (28.7 months vs. 11.3 months; HR, 0.47; CI, 0.31–0.72) as well as HRD-negative (12.1 months vs. 9.1 months; HR, 0.65; CI, 0.45–0.95) populations (8). Although there were some variations in the inclusion criteria in the above trials, both the tests predicted a higher magnitude of benefit in BRCA-mutated and HRD-positive than HRD-negative subgroups, as demonstrated by the HR. In the PAOLA-1 study, HRD assessment by the myChoice assay predicted benefit with olaparib maintenance in median PFS in HRD-positive (37.2 months vs. 17.7 months; HR, 0.33; CI, 0.25–0.45) but not in HRD-negative (16.6 months vs. 16.2 months; HR, 1.0; CI, 0.75–1.35) subgroups (9). In platinum-sensitive relapsed ovarian cancer, the myChoice HRD assay predicted greater benefit with niraparib in HRD-positive (HR, 0.38; CI, 0.23–0.63) than HRD-negative (HR, 0.58; CI, 0.36–0.92) subgroups in BRCA wild-type patients in the NOVA trial (10).

It is noteworthy that the mechanism of action of PARPi in HRD-negative patients remains incompletely elucidated and PARPi activity extends beyond HRD, likely in large part as patients are typically selected for treatment based on functional response to platinum-based chemotherapy (7–10). Further, HRD is dynamic process, and the effect of therapy may change its (repair) function and status. Genomic scars, however, are generally permanent and persist even if the initiating event is reversed (11). The development of resistance to PARPi, for example, through changes in drug efflux and replication fork stabilization, also limits its predictive value (12, 13). As a consequence, these tests do not accurately determine HRD status following progression on platinum or PARPi. In addition, the requirement for high tumor content and quality of formalin-fixed, paraffin-embedded (FFPE) tissue may also affect the accurate assessment of HRD. Therefore, ongoing efforts to improve the predictive value of HRD testing methods are critical to better identify patients who may benefit from PARPi therapy.

Functional assays, which can more accurately reflect a dynamic, real-time picture HRD, gene expression profiling, methylation, and protein expression are currently under development (14– 17). Functional HRD (fHRD) assessment are performed directly by either

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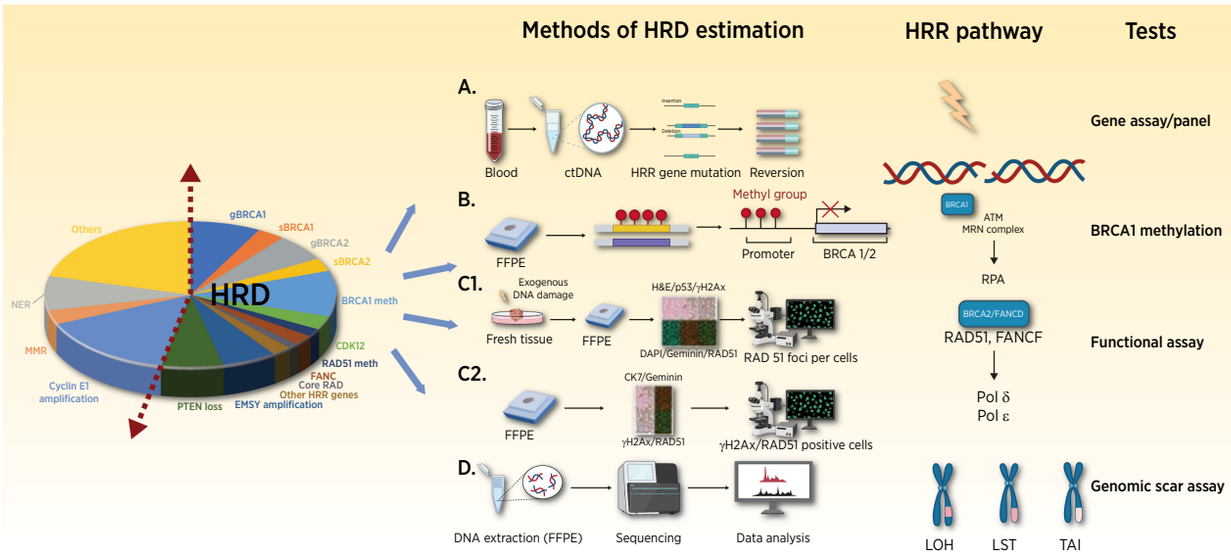

Figure 1.

Predictive methods of HRD assessment in ovarian cancer. **A,** Germline or somatic gene sequencing or panel testing of *BRCA1/BRCA2* or other HRR genes [BRACAnalysis CDx and MyRisk (Myriad Genetics), FoundationFocus CDxBRCA (Foundation Medicine)]. These have highest predictive value for response to PARPi therapy. Circulating tumor DNA (ctDNA) may be used to assess BRCA reversion mutations as well. **B,** *BRCA1* promoter methylation (BRCA1meth) causes epigenetic silencing of *BRCA1* gene function leading to HRD. Methylation is mutually exclusive to BRCA mutations and can be observed in 15% of patients with ovarian cancer. BRCA1meth can assess dynamic HRD status as loss of methylation may predict emergence of resistance to PARPis. **C,** Functional *RAD51* assays **C1.** RECAP test assesses exogenous radiation-induced DNA damage and requires fresh tumor cells. Irradiated tissue is fixed and stained with antibodies against hematoxylin and eosin, p53 (detect tumor cells), γH2Ax (assess DNA damage), DAPI (stains nuclear DNA and determine nuclei), geminin (marker of cells in S–G_2 phase), and RAD51 (signifies HRR). Fluorescence microscopy is used to quantify the level of RAD51 foci per cell to mark sites of active HRR. **C2.** FFPE-fHRD assay assesses endogenously damaged DNA in ovarian cancer cells. FFPE specimen immunostained with CK7 (to assess tumor cells), geminin, γH2Ax, and RAD51 to assess RAD51 loading on in S–G_2 phase of cell cycle. Nuclei positive for both geminin-γH2Ax and geminin-RAD51 measured to quantify HRD status. **D,** Genomic scar assays are validated assays [myChoice HRD (Myriad Genetics) and FoundationOne CDx assay (Foundation Medicine)] that detect permanent structural chromosomal abnormalities or genomic signatures that occur because of HRD viz., LOH, LST, and TAI. (Adapted from an image created with BioRender.com.)

studying patient-derived tumor organoids or indirectly using surrogates like *RAD51* loading, which has been widely studied in breast, and ovarian cancers (18). *RAD51* loading signifies proficiency of upstream HRR components starting with *BRCA1/2*, while loss of *RAD51* suggest alterations in the upstream pathways. Currently available tests include REcombination CAPacity (RECAP) test, RAD51-FFPE test, and qIHC assay. RECAP test involves exposing fresh tumor cells to ionizing radiation to induce DNA damage and then assessing cell's ability to repair the damage. The radiation exposed cells are fixed and stained with antibodies against RAD51 (signifies HRR) and geminin (marker of cells in S–G_2 phase) to mark sites of active HRR. The stained cells are analyzed using fluorescence microscopy to quantify the level of RAD51 foci per cell, which reflects the cell's ability to repair DNA damage by HRR. However, the requirement for fresh tumors and the need for *ex vivo* induction make its clinical application challenging. In addition, the *ex vivo* induction of DNA damage may introduce variability in the results, as the efficiency of DNA damage induction may vary between samples (19). van Wijk and colleagues optimized the protocol of RAD51-GMN immunofluorescence on FFPE tissue and calibrated results to matched RECAP test data (20). Hoppe and colleagues developed a qIHC assay to measure RAD51 expression levels in FFPE tissue and defined a RAD51 nuclear expression score (RAD51NES) based on the average expression of RAD51 across all imaged tumor cells. RAD51 expression was analyzed in ovarian cancer cohort on standard-of-care therapy, and further validated on the SCOTROC4 trial cohort treated with carboplatin monotherapy. High RAD51NES predicted platinum resistance as these patients had lower PFS and overall survival (OS; ref. 21).

In this issue, Pikkusaari and colleagues report a novel method of estimating *RAD51* using endogenous DNA damage in treatment-naïve as well as chemotherapy treated ovarian cancer. FFPE specimen were immunostained with CK7 (stain tumor cells), geminin (marker of cells in S–G_2 phase), γH2Ax (assess DNA damage), and RAD51 (signifies HRR) to assess RAD51 loading on endogenously damaged DNA in ovarian cancer cells in S–G_2 phase of cell cycle. S–G_2 phase nuclei are scored as either RAD51 positive or negative (not based on number of foci) and automated analyzers performed quantitative RAD51 assessment by detecting nuclei positive for both geminin-γH2Ax and geminin-RAD51. Different cutoffs were used for treatment-naïve (>10% of cells) and chemotherapy treated (>30% of cells) specimens as chemotherapy treated cells tend to have a higher DNA damage. On the basis of these, a functional homologous recombination score of <10.0 was identified for treatment-naïve and <30.0 for chemotherapy treated specimens to define fHRD or functional homologous recombination–proficient (fHRP). Results were correlated with clinical outcomes and compared with other HRD tests performed on the same specimens. This assay successfully obtained HRD scores from 94% samples, even from those with low tumor content. Although individual functional homologous recombination scores were higher in chemotherapy treated specimen compared with treatment-naïve samples, all paired samples (pre- and post-chemotherapy) had concordance of fHRD status. Among BRCAmut tumors, 88% were scored as fHRD,

Table 1. Differences between methods of HRD assessment in ovarian cancer.

Test	Technique	Advantages	Drawbacks
Gene sequencing or panel testing of *BRCA1/BRCA2* genes. BRACAnalysis CDx and MyRisk (Myriad Genetics), FoundationFocus CDxBRCA (Foundation Medicine)	Sequencing of HRR pathway genes	• Highest predictive value • Germline testing crucial for cancer risk in family members. • Widely available	• Misses HRD in >50% cases • VUS • Reversal mutations can restore HRR status.
Genomic Scar assay myChoice HRD (Myriad Genetics), FoundationOne CDx assay (Foundation Medicine)	Quantifies LOH, LST, and TAI by estimating CNV in SNP using NGS; hybrid capture NGS	• Predict response to PARPi • Validated in prospective studies • Detect HRD in vast majority (high-sensitivity and specificity)	• Reversal mutations can restore HRR status. • High cost • Misses some patients with HRD • Require high tumor content for accurate estimation (screen failure)
RECAP test	Estimation of RAD51 foci formation using IHC in proliferating cells after *ex vivo* irradiation of fresh tumor tissue	• fHRD detection • Can more reliably assess HRD status at relapse	• Requirement for fresh tumors and the need for *ex vivo* irradiation • Predictive value for PARPi unknown • Lack of prospective validation • Can miss alterations downstream or independent of RAD51
Functional RAD51 assay (1)	Immunostaining of FFPE specimens with CK7, geminin, γH2Ax, and RAD51	• Dynamic HRD detection • Low cost and does not require expensive equipment • Can be performed on post-chemotherapy samples with low tumor content • Predicts response to platinum therapy	• Predictive value for PARPi unknown • Lack of prospective validation • Can miss alterations downstream or independent of RAD51 • May be difficult on archival tissue (time to fixation <2 hours and presence of sufficient endogenous DNA damage)
BRCA promoter methylation assay (2)	Methylation-specific PCR assay or digital droplet PCR	• Dynamic HRD detection • Can help in detecting resistance mechanism to PARPi • Low cost • Easily reproducible	• Lack of prospective validation • Limited applicability as methylation seen in only 15% • Significance of low BRCA methylation unclear

Abbreviation: VUS, variant of unknown significance.

while the 12% tumors that had somatic *BRCA2* mutations had fHRP scores. These were found to have *BRCA2* reversion mutations when WGS performed on relapse specimens of these patients. Furthermore, fHRD specimens had higher proportion of complete and partial response to primary platinum-based therapy, and scores negatively correlated with PFS and OS. Although very few received PARPi therapy, fHRD status predicted longer survival with PARPi therapy. Only 40% of patients had concordance of HRD status according to all HRD methods.

This new fHRD assay is promising as it can be performed on pre- and post- chemotherapy samples (with low tumor content), thereby providing dynamic HRD monitoring. Furthermore, this assay is cost-effective and can be performed using a 20× microscope and does not require expensive equipment. In addition, the assay may detect subclonal BRCA reversion, which is a key feature in predicting therapeutic response. However, there are certain limitations in its ability to identify all fHRD samples. Specifically, it can only detect those with impaired RAD51 loading and may miss any alterations downstream or independent of RAD51. While the test failure rate was low, it also requires sufficient endogenous DNA damage in the S–G_2 phase of the cell cycle for accurate assessment. The time to fixation of less than two hours is necessary for successful HRD detection, which increases the likelihood of failure with archival tissues. This assay also predicted a response to platinum therapy in newly diagnosed ovarian cancer as greater responses were reported in fHRD, but its utility in predicting response to PARPi therapy needs further validation. While promising for detecting dynamic HRD, its value in predicting response to platinum and PARPi in in recurrent settings has yet to be demonstrated.

Blanc-Durand and colleagues performed quantitative analysis of BRAC1 promoter methylation (BRCA1meth) in stored samples using digital droplet polymerase chain reaction. BRCA1meth was quantified as ratio of allelic frequencies of BRCA1meth and TP53 mutation. Cut-off for high BRCA1meth (≥70%) which denotes homozygous silencing and low BRCA1meth (1%–69%) which denote monoallelic or partial silencing of BRCA1 were defined and compared with GIS (MyChoice Cdx). The BRCAmut and BRCA1meth specimens had significantly higher GIS scores compared with unmethylated ones. Presence of BRCA1meth (high or low) was 100% predictive of HRD by GIS (≥42). However, methylations status did not correlate with survival and about one fourth of samples with HRD by GIS were BRCA1 unmethylated. BRCA1meth can detect dynamic HRD status as methylation levels can be altered by treatment, and loss of methylation may predict resistance to therapy (22). In this study, five patients (all with high GIS) had low BRCA1meth after chemotherapy treatment and three among these relapsed early, while no relapses were observed in 6 patients with low BRCA1meth prior to chemotherapy treatment. Although pre-therapy methylation status was unknown, there is possibility of loss of methylation under influence of platinum therapy leading to emergence of

resistance. However, significance of low BRCA1meth remains unclear in treatment-naïve settings. Further, predictive relevance of BRCA1-meth remains debatable due to lack of consistent results (23, 24).

A robust predictive biomarker needs to be reliable (easily reproducible at local laboratories), have a high clinical validity (to minimize false negatives and false positive results), low screen failure rate and a quick turnaround time so that most patients can benefit from treatment and at an appropriate time. Further, it should be minimally invasive and cost-effective for universal applicability (25). Despite the availability of multiple HRD assays (**Table 1**), much work remains with clinical validation and utility especially in the setting of recurrent disease and platinum resistance. Although genomic scar assays have been prospectively validated, they are more susceptible to false negatives and screen failures and utility is limited in platinum resistant ovarian cancer. The cost of approved HRD assays can be a barrier, but the potential to accurately predict value from expensive therapies like PARPi could easily make these cost-effective. Dynamic tests such as the reported ones may extend predictive utility and would need to be coupled with real time tissue biopsy. The RAD51 functional assay shows promise as a potential real time HRD biomarker and future research should focus on refining and clinically validating its utility for treatment selection in prospective randomized clinical trials.

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