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Review Article

Homologous Recombination Repair Deficiency: An Overview for Pathologists

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ABSTRACT

The repair of DNA double-stranded breaks relies on the homologous recombination repair pathway and is critical to cell function. However, this pathway can be lost in some cancers such as breast, ovarian, endometrial, pancreatic, and prostate cancers. Cancer cells with homologous recombination deficiency (HRD) are sensitive to targeted inhibition of poly-ADP ribose polymerase (PARP), a key component of alternative backup DNA repair pathways. Identifying patients with cancer with HRD biomarkers allows the identification of patients likely to benefit from PARP inhibitor therapies. In this study, we describe the causes of HRD, the underlying molecular changes resulting from HRD that form the basis of different molecular HRD assays, and discuss the issues around their clinical use. This overview is directed toward practicing pathologists wishing to be informed of this new predictive biomarker, as PARP inhibitors are increasingly used in standard care settings.

Introduction

The ability of organisms to faithfully copy and repair DNA is a universal trait and critical to cell survival and duplication. Thus, multiple repair mechanisms have evolved to ensure the fidelity of DNA is retained. The most important is the ability to recover from breaks affecting both DNA strands simultaneously. Double-strand breaks (DSBs) can be repaired by 2 distinct processes in the cell: one that results in faithful repair and the other that does not. Genomic instability, resulting from reliance on the latter error-prone repair, is one of the hallmarks of cancer.[1] It is a common feature in many cancer types, such as breast, ovarian, endometrial, pancreatic, and prostate cancers.

Novel compounds have been discovered in the past 2 decades that inhibit the single-strand break repair enzyme poly-ADP ribose polymerase (PARP) and inhibit error-prone DSB repair.[2] Targeted therapies based on these compounds, such as olaparib, rucaparib, and niraparib, have shown clinical efficacy with prolonged survival in patients with cancers that exhibit a high degree of genomic instability. The concept underlying this response is termed synthetic lethality, whereby cells that have lost their ability to precisely repair DSBs must revert to error-prone repair. Conversely, normal cells exposed to PARP inhibitors (PARPi's) exhibit defective single-strand break repair and rely more heavily on high-fidelity DSB repair to remove these otherwise innocuous lesions. Thus, cells not only with intrinsic defects in high-fidelity DSB repair but also exposed to a PARPi tend to accumulate unrepaired DSBs that destabilize the genome to such an extent that cell death ultimately ensues. Two genes or proteins are synthetically lethal when inactivation of either one is compatible with cell viability but inactivation of both results in cell death.[3]

The aim of precision oncology is to ensure that a targeted therapy is given only to patients whose tumors harbor the "cognate" change, thereby ensuring maximal clinical benefit.[4] In

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Table 1
Glossary of terms

Glossary of Terms
Homologous recombination repair (HRR): A DNA repair through the homologous recombination repair pathway for the error-free repair of double-stranded DNA breaks using sister chromatids as a template.
Homologous recombination deficiency (HRD): A phenotype that is characterized by the inability of a cell to effectively repair DNA double-stranded breaks using the HRR pathway.[5]
Homologous recombination proficiency (HRP): The absence of HRD.
Reversion mutation: A mutation event that restores a wild-type phenotype, usually by altering or removing an allele that confers a mutant phenotype.
Copy number variation (CNV): A genomic segment of more than 50 bp that differs in the copy number based on the comparison of the germline genome of 2 or more individuals.
Somatic copy number alterations (SCNA)[6]: A genomic segment larger than 10 kb but smaller than a whole chromosome arm that differs in the copy number based on the comparison of 2 or more genomes and that occurs postzygotically (in a somatic cell).
Aneuploidy: All copy number alterations affecting whole chromosomes or entire chromosome arms (excluding the short arms of acrocentric chromosomes).
Mutational signatures: Patterns of mutations in the genome that arise from biological processes during tumorigenesis. These patterns may be because of past or ongoing exposure to mutagens such as radiation or chemicals or deficiencies in DNA repair pathways such as HRR.
Loss of heterozygosity (LOH): The somatic loss of 1 allele at a specific genetic locus. LOH is generally associated with a loss of genetic material and often constitutes a "second hit," leading to malignancy if the remaining allele harbors a nonfunctional tumor suppressor gene.
Large-scale transition (LST): Somatic alterations that generate regions of LOH of greater than or equal to 10 Mb.[7]
Telomeric allelic imbalance (TAI): Number of subchromosomal regions with LOH extending to the telomere.
Nonhomologous end joining (NHEJ): A pathway that repairs double-strand breaks in DNA. "Nonhomologous" refers to break end ligation without the use of a homologous template, in contrast to HRR. It is also referred as **microhomology-mediated end joining (MMEJ).**

the case of PARPi's, it is essential to identify tumors that demonstrate defective DSB repair to ensure these drugs are delivered to the correct patients. Table 1 presents the glossary of terms used.[5-7]

DNA Repair Mechanisms

DNA duplication before cell division normally occurs with very high fidelity—in the order of 1 nucleotide error per 10^9 nucleotides.[8] This high level of accuracy is critical to retaining the genome integrity in higher organisms. Five key pathways (Fig. 1) are responsible for the repair of DNA errors, but, particularly, 2 are responsible for the most critical of errors, DSBs. The repair of DSBs is particularly important during DNA replication when the double helix may be completely split apart (Fig. 2; an animated video of DNA damage and repair processes may also be found at https://vimeo.com/705985384 [9]). The 2 key DSB repair mechanisms—nonhomologous end joining (NHEJ) and homologous recombination repair (HRR) (Fig. 3)—differ in the key protein components involved, the source of the DNA template strand used, and the phases of the cell cycle during which they operate. NHEJ is a more straightforward but unreliable repair mechanism and is available throughout the cell cycle. This repair process uses microhomology (short strands of complementary DNA) between the 2 broken ends of DNA to repair the DSB, but repair is at the expense of the deletion of some nucleotides (nontemplated repair). NHEJ-induced deletions are likely to be inconsequential when occurring in noncoding regions but may have significant consequences in the ~2% of the genome harboring protein-coding genes, potentially resulting in reading frame alterations and the loss of gene expression.

HRR is the most high-fidelity pathway. It uses the complementary DNA strands of the nearby sister chromatid, present during the replication and growth phases of the cell cycle (S and G2), as a template to exactly repair the DSB. There are multiple steps in the process, starting with the detection of DSBs by the MRN complex[10] (Fig. 2). Next, exonucleases degrade the 5' DNA strand, a process requiring functional *BRCA1*. Finally, template strand invasion is facilitated by *BRCA2*, and strand synthesis occurs by exactly copying the intact sister chromatid sequence (Fig. 4).

Both NHEJ and HRR are used in other important cellular processes. During meiosis, both pathways are used to generate crossovers, a requirement for "gene mixing," which creates genetic diversity within a population. Moreover, the error-prone nature of NHEJ is harnessed in cells of the immune system when forming functional antibodies and T-cell receptors, which greatly increases DNA sequence diversity. Finally, the HRR pathway repairs DNA interstrand crosslinks that can prevent transcription and replication by inhibiting DNA strand separation.[11]

There are multiple genes[3] in the HRR pathway, including *BRCA1* and *BRCA2*, 2 of the most widely studied cancer genes, the MRN complex genes (*MRE11A*, *NBN*, *RAD50,* and *ATM*), and other genes involved in strand invasion (*PALB2* and *RAD51*). Mutations in any of these genes may result in the phenotype of homologous recombination deficiency (HRD).[12] In addition to the loss of function mutations, epigenetic changes such as the methylation of HRR gene promoters may disable these genes and lead to the HRD phenotype.[13] Because affected tumor cells are no longer able to rely on the HRR pathway for repairing DSBs, they fall back on the alternate pathway for repair, NHEJ. The error-prone nature of NHEJ leads to characteristic "genomic scars" that are a tell-tale sign identifying tumors with HRD irrespective of the underlying etiology (whether genetic or epigenetic). This scarring has been termed BRCAness, and its measurement has been the focus of much cancer genome research in recent years.

Measuring HRD

Because of the high error rate inherent in the NHEJ pathway, cells with HRD accumulate mutations that contribute to the development of cancer in a process analogous to that whereby cells with defective mismatch repair genes, being unable to maintain microsatellite stability, acquire damaging mutations at a higher rate than healthy cells.[14] In the case of defective mismatch repair genes, microsatellite instability is a measurable outcome of the underlying "mutator phenotype" and is now an approved biomarker for the identification of patients likely to benefit from immunotherapy.[15] Moreover, it is possible to measure the "genomic scars" that occur in HRD cells and use these as

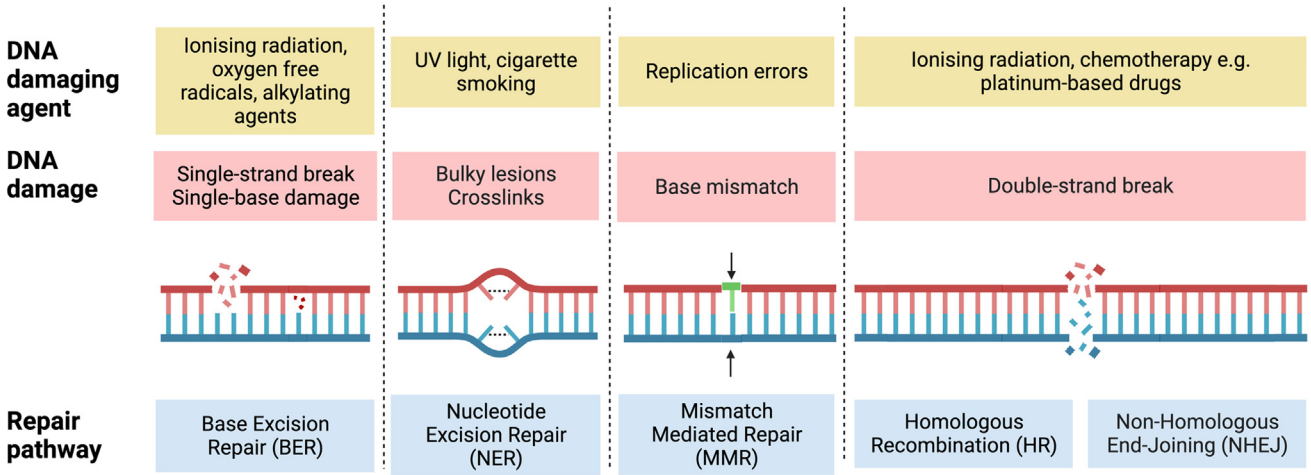

Figure 1.
Mechanisms of DNA damage, their causes, and their repair pathways.

biomarkers for predicting response to targeted PARPi therapy. These HRD detection methodologies are discussed further.

Genomic Scars of HRD

The genome instability in HRD tumors causes a range of genetic abnormalities of varying sizes that are detectable by current sequencing technologies. Large structural variations of 0.1 to 10 Mb in size and interchromosomal rearrangements occur at the chromosome scale. At the gene scale, deletions and tandem duplications less than 100 kb with detectable flanking microhomology occur. Events at both these scales contribute to a high rate of somatic copy number alterations (SCNAs) in HRD tumors. At the nucleotide scale, single base substitutions and indels contribute to the characteristic mutational signature further described.

Historically, SCNA has been the target of choice for scarring assays, and measurement has most often been achieved by quantitating loss of heterozygosity (LOH) on high-density SNP arrays. Popova et al[16] measured LOH by this method and defined events termed "large-scale transitions" (LST), chromosomal breaks between adjacent regions of at least 10 Mb, whose number correlated with *BRCA* mutation status in basal-like breast cancers. Similarly, the magnitude of "telomeric allelic imbalance" (TAI), SNP array–defined regions of copy number loss extending to telomeres, was seen to be associated with cisplatin sensitivity in triple-negative breast cancer in the study by Birkbak et al.[17] Finally, Abkevich et al[18] used single nucleotide polymorphism (SNP) arrays to define and count the number of LOH regions of >15Mb in high-grade serous carcinoma and found that the number correlated with mutational loss or epigenetic silencing of *BRCA1*, *BRCA2*, or *RAD51C* (see Table 1 for definitions). These features can be used singly or combined to provide a metric of genomic instability that reflects the extent of HRD in tumors.[19] Table 2 summarizes the indicators and assays available for HRD.

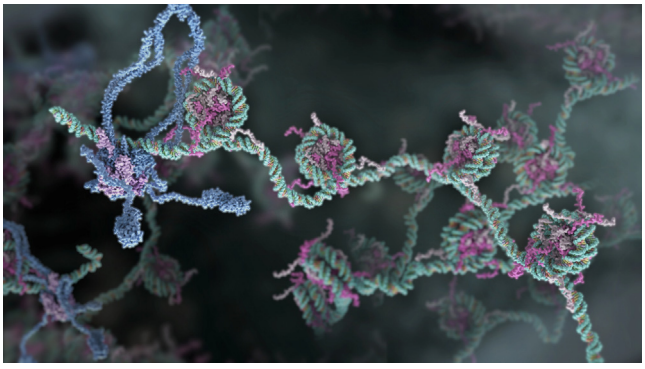

Figure 2.
Still frame from the animation "*DNA Damage and Repair*" (https://vimeo.com/705985384). The MRN complex (blue and light purple) finding a double-stranded DNA break.[9]

Mutational Signatures of HRD

Multiple endogenous and exogenous processes damage and repair a cells genome over its lifetime.[20] Some of these processes result in characteristic patterns of alterations, which can be classified according to the combination and frequency of recurrent nucleotide triplets (a mutated base and its 2 adjacent nucleotides) identified across the tumor genome. Currently, 49 so-called mutational signatures have been observed in cancers, many of which have been attributed to a specific mutational process.[20,21] Relevant to detecting HRD, signature 3 is associated with *BRCA1* or *BRCA2* loss. Mutational signatures are usually measured using whole genome sequencing (WGS) or whole exome sequencing (WES). However, the ESMO recommendations for ovarian cancer[13] concluded, "there is currently insufficient evidence to ascertain the clinical validity of WGS-based mutational signatures for predicting PARPi benefit in HGSC." However, recent bioinformatic tools, such as SigMA,[22] are now able to detect signature 3 using sequence data from large targeted panels that are routinely used in many clinical contexts.

Loss of the HRD Phenotype

One caveat exists for most scarring assays—the historical nature of these events may disguise the current HR status of the tumor. Prolonged treatment with platinum-based or PARPi therapies can result in the selection of resistance-causing reversion mutations or HRR gene reactivation by promoter demethylation.[23,24] In such circumstances, although the genomic scarring and

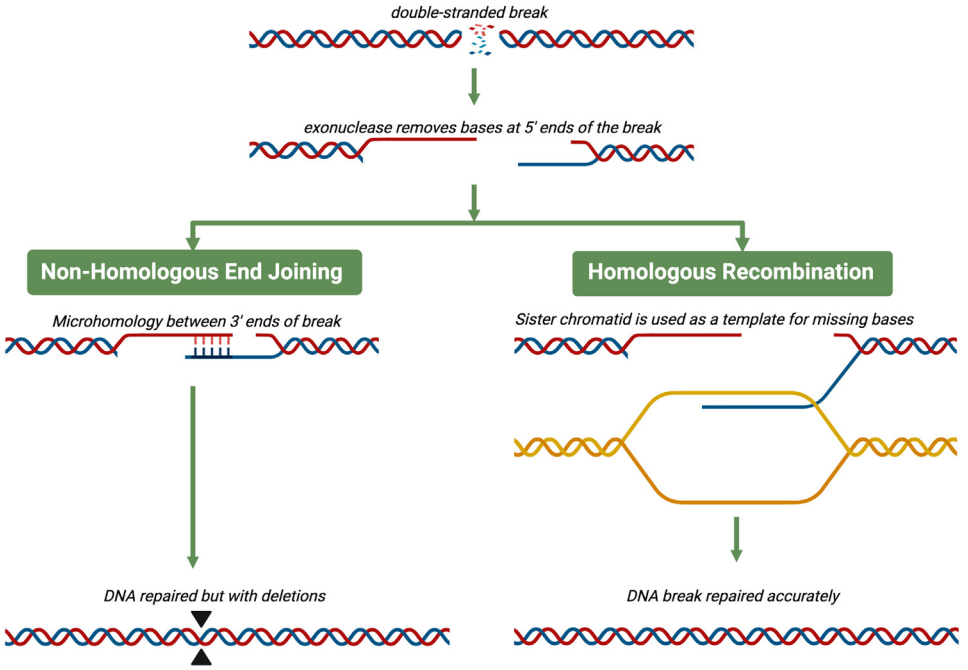

Figure 3.
DNA double-stranded break repair mechanisms. Nonhomologous end joining leaves evidence of its activity on the genome in the form of deletions flanked with a small number of homologous bases (microhomology). By contrast, no traces are left by the homologous recombination repair pathway.

mutational signatures of HRD may still be evident in the relapsed disease, these tumor cells have most likely sufficiently recovered their HRR ability to make the ongoing use of PARPi ineffective.

Functional Assessment of HRD

To directly test HRR proficiency, there has been some work to develop functional assays in contrast to the above-discussed more indirect indicators. Although not in clinical use, one such method estimates the amount of nuclear *RAD51*, the DNA recombinase involved in template strand invasion. However, this *RAD51* assay has multiple limitations[13]: (1) it requires immunofluorescence, which many laboratories do not support; (2) it is laborious, requiring *RAD51* foci quantitation in 40 cells by a pathologist; (3) any defects in pathway components downstream of *RAD51*'s involvement are not detected; and (4) radiation is used to induce exogenous DNA damage and a *RAD51* signal for the assay. All these limitations make this assay impractical in a clinical setting. Nonetheless, refinement of the assay using standard formalin-fixed paraffin-embedded (FFPE) samples has been accomplished, potentially making it an attractive tool for clinical laboratories as a future functional homologous recombination proficiency assay, with a simpler workflow and a sensitivity of 90% in *BRCA*-deficient tumors.[25,26] Sample choice is a critical factor when performing functional assays. Thus, archival tissue collected at surgical resection may not be representative of the treated patient's current HRR status because of the acquired resistance discussed earlier.

HRD Assays

At the time of writing, 4 commercial HRD assays are available, 2 of which are approved by the U.S. Food and Drug Administration

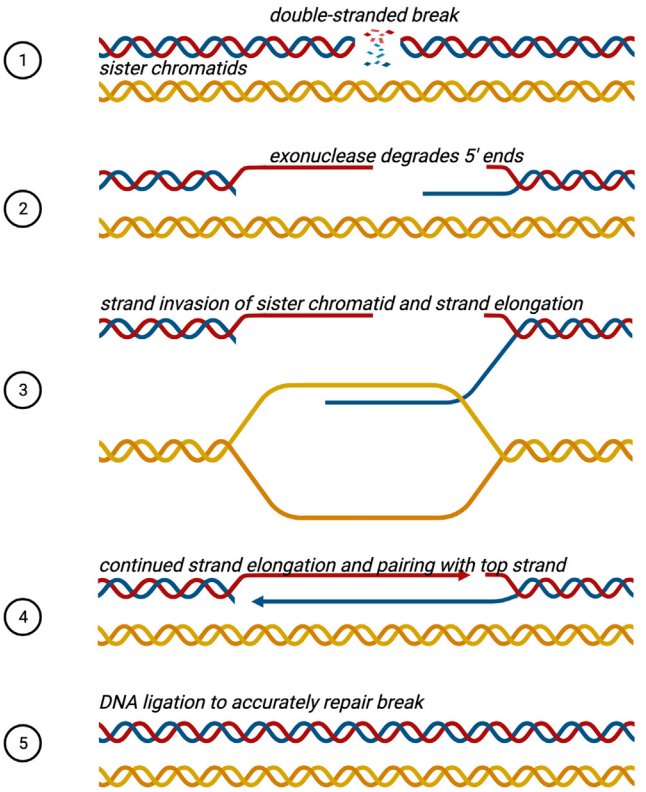

Figure 4.
Steps in homologous recombination repair using templated repair. (1) A DNA double-stranded break occurs. (2) Some bases at the 5' ends of the break strands are removed. (3) One DNA strand invades the sister chromatid and is paired by homology. The intact strand elongates past the break point. (4) Strands complete elongation and repair with original chromatid. (5) DNA ligation completes the join, and repair is completed faithfully.

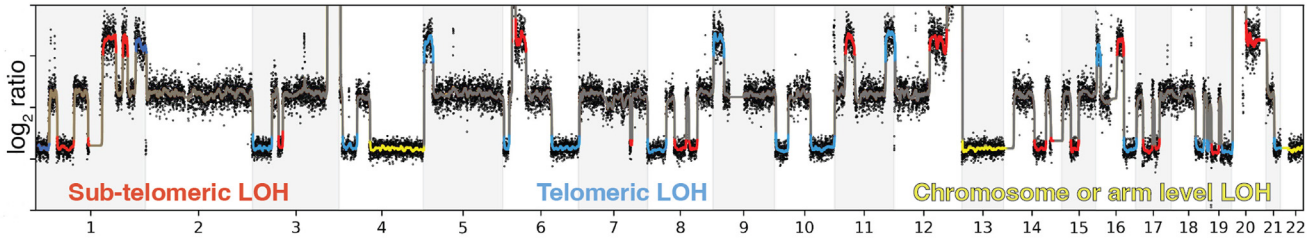

Figure 5.
Genomic loss of heterozygosity (LOH) scoring as performed by panels such as Myriad myChoice CDx, FoundationCDxBRCA, and AmoyDx. Contiguous regions of LOH are counted toward the overall score unless they extend to a whole chromosome or arm (yellow regions). FoundationCDxBRCA is positive if ≥16% of scored SNPs fall into telomeric (blue) or subtelomeric (red) LOH regions. AmoyDx and Myriad myChoice apply proprietary weighing to telomeric vs subtelomeric LOH events.

for certain drug indications, together with some research assays based on WGS or WES (Table 2). The 2 assays that are Food and Drug Administration approved as companion diagnostics for ovarian cancer are Foundation Medicine's FoundationFocus CDxBRCA LOH and Myriad's myChoice HRD, which are approved for selecting patients for rucaparib and olaparib, respectively.[27,28] All assays identify both HRR gene mutation status and the large-scale genomic changes resulting from HRD scarring. Key differences include the following: whether only *BRCA1/2* gene mutations are assessed or an expanded panel of HRR genes and whether the assay is a targeted panel or requires WGS and the algorithm to generate a genomic HRD scarring index (Table 2). Notably, although none of the assays directly assess epigenetic silencing of HRR genes, these events undoubtedly account for some of the cases that are negative for HRR gene mutation (~50% of ovarian carcinomas) but have a positive HRD score.

Differences in the approach for the detection of genomic scars and the corresponding (often proprietary) scoring algorithm are significant between these assays (Fig. 5). As discussed earlier, many assays use loss of genetic markers as a proxy measure of SCNAs, with LOH at well-characterized SNP positions being the marker of choice. Originally developed for high-density SNP microarrays, LOH is now more commonly determined by next generation sequencing (NGS)-based SNP genotyping. Other assays use normalization methods to infer copy numbers directly from sequencing read depth. Although usually in agreement, genotyping and read-counting methods may not always coincide. Moreover, differences between sample cohorts used to train and validate these algorithms may result in disagreement between the assays in assigning HRD status. For recent comparisons, refer the studies by Stewart et al[5] and Miller et al.[13]

Foundation Medicine CDxBRCA LOH Assay

The Foundation Medicine CDxBRCA LOH[27] assay is a targeted NGS assay that assesses FFPE tumor samples for mutations in *BRCA1/2*, other HRR genes, and genomic LOH. A positive HRD status is defined as tumor *BRCA* positive and/or "high" LOH status. The FoundationOne CDx assay incorporates the CDxBRCA assay and provides HRD status, microsatellite instability and tumor mutational burden status, and the mutational status of 324 cancer genes, including the major HRR genes. The genomic LOH score is determined by inferring the percentage of focal LOH in the tumor by genotyping a large (but unspecified) number of SNPs across the genome. A genomic LOH score of ≥16% is regarded as "LOH high."

Table 2
HRD assays described in the text are summarized with their biomarkers.

	Company or patent holder	Myriad Genetics	Foundation Medicine	SOPHiA Genetics	AmoyDx	Nik-Zainal and colleagues	Utrecht University Medical Centre	Harvard Medical School
	Name	myCHOICE CDx Plus	Foundation Focus CDx BRCA	Homologous Recombination Solution	HRD Focus Panel	HRDetect	CHORD	SigMA
Assays	Clinical Indication	Breast, Ovarian	Ovarian	Ovarian	Breast, Ovarian	Breast, Ovarian, Pancreatic	Pan-cancer	Pan-cancer
	Specimen	FFPE	FFPE	FFPE	FFPE	Fresh/frozen	FFPE	FFPE
	Regulatory Status	CDx	CDx	RUO	IVD	LDT	LDT	LDT
Biomarkers	Genomic scarring	LOH[a]	LOH[b]	—	LOH[c]	Flanking micro-homology	Flanking micro-homology	—
	Copy number analysis	—	—	lpWGS	—	WGS	WGS	—
	HRR gene mutations	*BRCA1*/2 + 13 genes	*BRCA1*/2	*BRCA1*/2 + 26 genes	*BRCA1*/2	Whole genome	Whole genome	≥300 gene panel
	Base substitution signatures	—	—	—	—	Sig 3, Sig 8	—	Sig 3
	Rearrangement signatures	—	—	—	—	Sig 3, Sig 5	Sig 3, Sig 5	—

FFPE, formalin-fixed paraffin-embedded; HRD, homologous recombination deficiency; IVD, in-vitro diagnostic; LOH ,loss of heterozygosity; LDT, laboratory developed test; RUO, research use only; WGS, whole genome sequencing.
Available assays may use a variable, weighted, and often proprietary combination of multiple genomic parameters to improve the accuracy of HRD prediction.
[a] 54,000 SNP backbone and a combined weighted score of LOH, TAI, and LST (see text).
[b] SNP resolution not published; percentage of LOH across genome, ignoring arm scale LOH.
[c] 24,000 SNP backbone; machine learning model incorporating LOH segment length, telomere/centromere proximity, and allelic association.

Myriad Genetics myChoice Assay

The Myriad myChoice CDx Plus assay is approved for FFPE samples from breast or ovarian cancer. It detects mutations in *BRCA1/2* and 13 other HRR genes and calculates a genomic instability score (GIS) using a weighted aggregate of LOH, LST, and TAI counts.[29] A positive HRD status is indicated by a deleterious or suspected deleterious mutation in *BRCA1/2* and/or a positive GIS status. As previously discussed, LOH, LST, and TAI are derived from genotyping SNPs distributed across the genome.[28] The weighting applied to each component to derive the final GIS is proprietary, and the assay is currently only performed under license to Myriad Genetics. Therefore, myChoice HRD is currently unavailable in many regions.

SOPHiA Genetics DDM HRD

The SOPHiA Genetics Homologous Recombination Solution detects mutations in 28 HRR pathway genes by targeted NGS and is combined with low-pass WGS to identify large-scale copy number changes indicative of an HRD scar. The HRD signature is computed by a trained convoluted neural network algorithm. The output is a genomic instability index that is highly correlated with the Myriad myChoice GIS in independent studies.[30] Low-pass WGS efficiently identifies SCNA compared with LOH-based methods because the sequencing is randomly distributed across the genome, whereas SNP genotyping requires targeting sequencing of up to 50,000 specific loci. The results of both the mutational and scarring analyses are accessed in SOPHiA Genetics' cloud-based SOPHiA DDM platform, which facilitates interpretation and reporting. Although the assay itself is currently for research use only, it can potentially be performed clinically by locally accredited molecular pathology laboratories and reported using DDM, which is CE-IVD-compliant software. It supports both FFPE and fresh frozen material in quantities compatible with pathology laboratory use.

Amoy HRD Focus Panel

A commercial panel is available from Amoy, which assesses the HRD status in FFPE breast or ovarian cancer samples by examining *BRCA1/2* genes and a genomic scar score (GSS). A positive HRD status is given in the presence of a pathogenic/likely pathogenic mutation in *BRCA* genes or a positive GSS status (GSS $\geq$ 50.0). The GSS status is assessed by genotyping SNPs distributed evenly across the genome and calculating a weighted value of observed copy number events. For each event, the score combines the site, length, and type a copy number variant using a machine learning classifier. This assay can potentially be performed by locally accredited molecular pathology laboratories using cloud-based software for reporting.

Bioinformatic solutions

The following methods are not clinical assays as such but bioinformatic algorithms that analyze and classify data derived from large panel sequencing, WES, or WGS and are largely used in research contexts. Moreover, they tend to rely on fresh frozen tissue samples derived from variable tumor types, such as breast, ovarian, or pancreatic cancer.

HRDetect

A partly signature-based HRD algorithm, HRDetect, was developed by Dr Nik-Zainal and colleagues.[31,32] It combines 6 genomic features (deletions with microhomology, mutation signatures 3 and 8, rearrangement signatures 3 and 5, and HRD index) and generates a score using different weightings for each parameter. It performs well (sensitivity, 98.7%), relative to other HRD indicators such as GISs (sensitivity 60%),[28] but is limited to WGS input.[31]

CHORD

A machine learning classifier named CHORD[33] has been developed that uses a simplified set of genomic features (compared with HRDetect), which are used by a random forest[34] classification model to predict the presence of HRD. Similar to HRDetect, CHORD uses WGS but has the advantage of being tissue agnostic and can be used for any cancer type. Training features include single nucleotide base changes (6 possibilities), indels with flanking microhomology, indels in repeat regions, and structural variants stratified by length and type. CHORD performed well on 2 pan-cancer data sets independent of the training data set (>0.98 area under the ROC curve). The Hartwig Medical Foundation[35] DNA Analysis report uses CHORD as part of its analysis pipeline. Adapting this assay for a targeted panel would be a promising direction of development to make the classifier more widely applicable.

SigMA

The bioinformatic tools that identify genomic changes indicative of HRD generally rely on WGS and WES because they require many mutational events to ensure confident calls. Nevertheless, a computational tool named SigMA[22] has been developed by the Harvard Medical School that is able to detect mutational signature 3 from assays using relatively small targeted panels with relatively low absolute mutational counts. This tool uses machine learning techniques to classify the presence or absence of signature 3 by assessing the spectrum of observed mutations against the expected tumor-type specific signatures. It is noteworthy that the simpler genomic features used by the pan-cancer tool CHORD[33] achieves similar results and bypass the complexities of fitting sample mutational data[32] to mutational signatures.

Issues Using HRD Testing in the Clinic

A key issue with the commercial HRD assays is their conflicting definitions of what is an HRD-positive tumor. In particular, the algorithms for assessing genomic scarring by SNP genotyping across the tumor genome vary significantly between assays, primarily because of the different algorithms being trained on a variety of data sets and cancer types. The differing assay methodologies may result in discordant findings for the same patient sample. This lack of a consensus for HRD definition forces pathology laboratories to select an assay and present patient results in the context of the specific test performed and how the results were interpreted. A number of different studies have been used to validate these assays, but there is a lack of harmonization between the cohorts, cancer types, therapy status, and GSS cutoffs,

which makes consistent use problematic. Excellent reviews of the validation studies have been conducted by ESMO for ovarian cancer[13] and by Friends of Cancer Research for ovarian, breast, prostate, and pancreatic cancers.[5] Both reviews highlight the lack of consensus for HRD test definition and metrics. As highlighted earlier, the available assays vary in the tumor types for which they have been validated, and there is an urgent need for an international effort to define the harmonized interpretation of clinical assays for HRD measurement. The effort would be analogous to working groups that have harmonized the normal range for *BCR::ABL* quantitative PCR assays and tumor mutational burden assessments.

The Friends of Cancer Research HRD Harmonization Project supports using HRD biomarkers and is "currently examining sources of variability across HRD tests and identifying opportunities for alignment while proposing solutions to improve agreement in the future."[36]

The clinical benefit of HRD assays is focused on the stratification of patients into responders and nonresponders of PARPi. However, current tests lack negative predictive values. They are poor at identifying patients who will not benefit from PARPi therapies.[13] A particular driver of PARPi nonresponse is the existence of reversion mutations, as discussed earlier. In the future, the use of genomic methods with improved sensitivity to these mutational events and the use of functional assays such as the *RAD51*-foci assay may improve the negative predictive values.

As with all NGS diagnostics, the analytical performance of HRD assays is sensitive to sample tumor purity (the proportion of tumor cells relative to normal cells) and tumor heterogeneity (the range of tumor cells contributing to the HRD phenotype relative to other tumor cells). Low tumor purity and/or high heterogeneity increase the difficulty of correctly identifying HRD biomarkers such as pathogenic mutations, genomic scarring, and mutational signatures.[31]

Clinical assay costs, laboratory turnaround times, and the widespread availability of FFPE samples continue to drive targeted panels as the preferred assay type. This leads to compromises in the quality of HRD panel assays relative to a more complete WGS HRD assay. Ongoing research continues to refine the capabilities of panel assays, but further work is required to incorporate current knowledge into low-cost, clinically appropriate HRD assays.

Conclusion

Minimum standards for the assessment of HRD are yet to emerge for clinical use. In the meantime, individual laboratories must establish their own protocols within the constraints of commercial HRD tests and clearly document their choices in clinical reports. There is a need for a more accurate HRD test that fully exploits current knowledge of the HRR genes and pathway, reversion mutations, and large-scale signatures and can give a real-time reading of homologous recombination proficiency across all cancer types. Validated tests that accurately assess patients with *BRCA1/2* wild type and impaired HRR function need to be developed to not only identify this cohort who could benefit from PARPi therapies but also correctly identify patients who will derive no benefit.

As always, cost-effective assays suitable for clinical use will ensure the benefits of diagnostic testing are more widely accessible.

Author Contributions

S.F. conceived the study, and K.D. wrote the manuscript with significant contributions from A.F. and S.F. All authors read and approved the final manuscript.

Data Availability

Not applicable.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

Animated Images

This manuscript contains an image (Fig. 2) that can be animated using the Artivive Application.

Ethics Approval and Consent to Participate

Not applicable.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646–674.
2. Skalitzky DJ, Marakovits JT, Maegley KA, et al. Tricyclic benzimidazoles as potent poly(ADP-ribose) polymerase-1 inhibitors. *J Med Chem*. 2003;46:210–213.
3. Lord CJ, Ashworth A. BRCAness revisited. *Nat Rev Cancer*. 2016;16:110–120.
4. Garraway LA, Verweij J, Ballman KV. Precision oncology: an overview. *J Clin Oncol*. 2013;31:1803–1805.
5. Stewart MD, Merino Vega D, Arend RC, et al. Homologous recombination deficiency: concepts, definitions, and assays. *Oncologist*. 2022;27:167–174.
6. Harbers L, Agostini F, Nicos M, et al. Somatic copy number alterations in human cancers: an analysis of publicly available data from The Cancer Genome Atlas. *Front Oncol*. 2021;11:700568.
7. Schonhoft JD, Zhao JL, Jendrisak A, et al. Morphology-predicted large-scale transition number in circulating tumor cells identifies a chromosomal instability biomarker associated with poor outcome in castration-resistant prostate cancer. *Cancer Res*. 2020;80:4892–4903.
8. Alberts B. Molecular Biology of the Cell. In: *Garland Science*. 6th ed. Taylor and Francis Group; 2015.
9. Divjak M. Image/Animation credit: Dr Maja Divjak, Peter MacCallum Cancer Centre; 2021. Accessed May 2022. https://vimeo.com/majadivjak
10. Dzikiewicz-Krawczyk A. The importance of making ends meet: mutations in genes and altered expression of proteins of the MRN complex and cancer. *Mutat Res*. 2008;659:262–273.
11. Deans AJ, West SC. DNA interstrand crosslink repair and cancer. *Nat Rev Cancer*. 2011;11:467–480.
12. Heeke AL, Pishvaian MJ, Lynce F, et al. Prevalence of homologous recombination-related gene mutations across multiple cancer types. *JCO Precis Oncol*. 2018;2018. PO.17.00286.
13. Miller RE, Leary A, Scott CL, et al. ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. *Ann Oncol*. 2020;31:1606–1622.
14. Loeb LA. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat Rev Cancer*. 2011;11:450–457.

15. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18:1182–1191.
16. Popova T, Manie E, Rieunier G, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res*. 2012;72:5454–5462.
17. Birkbak NJ, Wang ZC, Kim JY, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discov*. 2012;2:366–375.
18. Abkevich V, Timms KM, Hennessy BT, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer*. 2012;107:1776–1782.
19. Timms KM, Abkevich V, Hughes E, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res*. 2014;16:475.
20. Alexandrov LB, Kim J, Haradhvala NJ, et al. The repertoire of mutational signatures in human cancer. *Nature*. 2020;578:94–101.
21. Degasperi A, Zou X, Amarante TD, et al. Substitution mutational signatures in whole-genome-sequenced cancers in the UK population. *Science*. 2022;376:abl9283.
22. Gulhan DC, Lee JJ, Melloni GEM, et al. Detecting the mutational signature of homologous recombination deficiency in clinical samples. *Nat Genet*. 2019;51:912–919.
23. Christie EL, Fereday S, Doig K, Pattnaik S, Dawson SJ, Bowtell DD. Reversion of BRCA1/2 germline mutations detected in circulating tumor DNA from patients with high-grade serous ovarian cancer. *J Clin Oncol*. 2017;35:1274–1280.
24. D'Andrea AD. Mechanisms of PARP inhibitor sensitivity and resistance. *DNA Repair (Amst)*. 2018;71:172–176.
25. Castroviejo-Bermejo M, Cruz C, Llop-Guevara A, et al. A RAD51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. *EMBO Mol Med*. 2018;10:e9172.
26. van Wijk LM, Kramer CJH, Vermeulen S, et al. The RAD51-FFPE Test; calibration of a functional homologous recombination deficiency test on diagnostic endometrial and ovarian tumor blocks. *Cancers (Basel)*. 2021;13:2994.
27. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31:1023–1031.
28. Telli ML, Timms KM, Reid J, et al. Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. *Clin Cancer Res*. 2016;22:3764–3773.
29. myChoice HRD Technical Specifications Effective Date: June 2017, Myriad Genetics, Accessed May 2022. https://myriad-web.s3.amazonaws.com/myChoice/downloads/myChoiceHRDTechSpecs.pdf
30. Buisson A, Saintigny P, Pujade-Lauraine E, et al. A deep learning solution for detection of homologous recombination deficiency in ovarian cancer using low pass whole-genome sequencing: evaluation of the analytical performance. *J Clin Oncol*. 2022;40:e17599.
31. Davies H, Glodzik D, Morganella S, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med*. 2017;23:517–525.
32. Degasperi A, Amarante TD, Czarnecki J, et al. A practical framework and online tool for mutational signature analyses show inter-tissue variation and driver dependencies. *Nat Cancer*. 2020;1:249–263.
33. Nguyen L, WM Martens J, Van Hoeck A, Cuppen E. Pan-cancer landscape of homologous recombination deficiency. *Nat Commun*. 2020;11:5584.
34. Ho TK. *Random Decision Forests*. AT&T Bell Laboratories; 1995.
35. Hartwig Medical Foundation. Accessed March 30, 2022. https://www.hartwigmedicalfoundation.nl
36. The Friends of Cancer Research—HRD Harmonization Project. Accessed September 21, 2022. https://friendsofcancerresearch.org/hrd