Review

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Review

Homologous Recombination Deficiency in Ovarian and Breast Cancers: Biomarkers, Diagnosis, and Treatment

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Abstract: Homologous recombination deficiency (HRD) is a pivotal biomarker in oncology, influencing treatment strategies and prognostic assessments. HRD impairs DNA repair mechanisms, leading to genomic instability and heightened sensitivity to poly(ADP-ribose) polymerase (PARP) inhibitors, particularly in ovarian and breast cancers. This review examines the molecular basis of HRD, current methodologies for its detection, and its clinical relevance in precision oncology. We address challenges in standardizing HRD assessment, including variability in testing approaches and interpretation thresholds. Additionally, emerging strategies for optimizing HRD-targeted therapies are discussed, emphasizing novel biomarkers and therapeutic combinations. By consolidating current knowledge and advancements, this review provides insights into HRD's role in cancer biology and its potential for refining personalized treatment approaches.

Keywords: Homologous recombination deficiency; DNA repair; genomic instability; PARP inhibitors; cancer biomarkers; precision oncology

1. Introduction

Homologous recombination deficiency (HRD) is a critical concept in cancer biology that has gained prominence in precision oncology due to its therapeutic implications [1,2]. HRD refers to a state where cells cannot effectively repair double-strand DNA breaks using the homologous recombination repair (HRR) pathway, a precise mechanism that relies on a homologous DNA template to restore genomic integrity. HRD gained prominence with the discovery that mutations in the breast cancer genes BRCA1 (Breast Cancer Gene 1) or BRCA2 (Breast Cancer Gene 2) impair homologous recombination repair (HRR) [3–5]. These BRCA1/2 mutations, linked to hereditary breast and ovarian cancers, lead to a distinct cancer phenotype. The complexity of HRD extends beyond just *BRCA*1/2 mutations, as it can arise from alterations in other HRR-related genes (e.g., *ATM*, *RAD51*, *PALB2*) or epigenetic changes, leading to a broader "HRD phenotype." HRD undermines the functionality of both double-strand DNA break repair (DSBR) and synthesis-dependent strand annealing (SDSA), leading to profound genomic instability. This understanding is vital for developing targeted therapies that capitalize on the DNA repair deficiencies in HRD-positive cancers [1,2,5].

Historically, HRD has been linked to increased sensitivity to DNA-damaging agents like platinum-based chemotherapy and targeted therapies such as poly (ADP-ribose) polymerase (PARP) inhibitors, which exploit synthetic lethality by blocking base excision repair (BER) and overwhelming HR-deficient cells with unrepaired DSBs [2,5]. The clinical relevance of HRD emerged prominently in cancers such as ovarian, breast, pancreatic, and prostate cancer, where identifying HRD-positive

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patients can guide treatment decisions [6]. However, there are ongoing challenges in defining HRD consistently, as its scope has expanded beyond *BRCA*1/2 mutations to include a wider array of genetic and functional defects, necessitating robust diagnostic tools and standardized criteria.

The review provides a comprehensive overview of HRD, bridging its biological basis, prevalence, and clinical relevance in ovarian and breast cancers. It offers updates and highlights from recent studies, discusses methods for HRD analysis and estimation, including genomic assays and functional tests, and addresses diagnostic challenges, while calling for further research to refine its assessment and application in oncology. Key points include the therapeutic implications of HRD (e.g., sensitivity to PARP inhibitors and platinum-based chemotherapy), the need for reliable, standardized assays to define HRD thresholds, and the necessity of integrating clinical, genomic and functional data for optimal patient stratification in ovarian and breast cancers.

2. HRD Pathway

Homologous recombination (HR) is a critical, high-fidelity DNA repair pathway that uses a homologous DNA sequence (typically from a sister chromatid) as a template to repair double-strand breaks (DSBs) to maintain genomic stability. This process is most active during the S and G2 phases of the cell cycle, when sister chromatids are readily available [7]. The homologous recombination (HR) pathway, a critical mechanism for double-strand DNA break repair (DSBR), initiates with the precise recognition of the lesion by sensor proteins, notably the MRN complex (MRE11-RAD50-NBS1) [7]. Following this detection, end resection, mediated by proteins such as CtIP and EXO1, generates 3' single-stranded DNA (ssDNA) tails, which are essential for strand invasion [8]. Subsequently, *RAD51*, a key recombinase, coats these ssDNA tails, facilitating strand invasion, where one tail pairs with the homologous DNA duplex, typically the sister chromatid, forming a displacement loop (D-loop) [9]. The D-loop, created by single-end invasion (SEI), may enter the DSBR pathway and form a double Holliday junction, where the invading strand and the homologous DNA are crossed over [10,11]. This invasion allows DNA polymerase to utilize the undamaged strand as a template to accurately repair the break. The process culminates in Holliday junction resolution, which can result in either crossover or non-crossover products, ultimately completing the repair [12]. This intricate and highly regulated pathway ensures genomic stability by faithfully restoring DNA integrity, thereby minimizing the accumulation of deleterious mutations.

Homologous recombination (HR) is not a singular, uniform process; it encompasses variations, most notably the double-strand break repair (DSBR) pathway and synthesis-dependent strand annealing (SDSA), which lead to distinct outcomes [13,14]. The DSBR pathway involves the formation of Holliday junctions, which are crucial intermediate structures formed when two homologous DNA duplexes are linked by crossed strands during the repair process [15,16]. Resolution of these Holliday junctions involves the cleavage of these cross-stranded links, carried out by Holliday junction resolvases. The manner in which resolvases cut the DNA strands determines whether the outcome of resolution is crossover (CO) or non-crossover (NCO) products [12,16]. Resolution to CO requires symmetric cleavage of both Holliday junctions in opposite orientations by a resolvase, while resolution to NCOs can also be achieved by the resolvase through cleavage of both junctions in the same orientation. Crossover events result in the exchange of genetic material between homologous chromosomes, as segments of DNA from one molecule are swapped with corresponding segments from the other. In SDSA, however, the process involves strand invasion and DNA synthesis, but the newly synthesized strands are displaced and anneal to the other broken end, without forming Holliday junctions [17,18]. This pathway primarily results in non-crossover products. DSBR, a robust repair mechanism, can contribute to genetic diversity through crossover events, which are particularly important during meiosis. However, the potential for crossovers means it can also lead to loss of heterozygosity. SDSA is a preferred pathway for maintaining genetic stability, as it avoids crossover events [17–19]. It is crucial for accurate DNA repair in somatic cells, where minimizing genetic rearrangements is essential, and the parental arrangement of genes is preserved. The choice between DSBR and SDSA depends on the cellular context, such as the cell cycle phase and the type of cell (somatic vs. germline). During meiosis, DSBR is crucial for generating genetic diversity in gametes [20]. In somatic cells, SDSA is often favored to preserve genomic integrity. Thus, SDSA

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promotes genomic stability, while DSBR has the potential to alter the genetic makeup of the cell [10,11,20,21].

Table 1. A summary of the key differences between the Double-Strand Break Repair (DSBR) and Synthesis-Dependent Strand Annealing (SDSA) pathways in homologous recombination:.

Feature	DSBR (Double-Strand Break Repair)	SDSA (Synthesis-Dependent Strand Annealing)
Holliday Junctions	Involves the formation of Holliday junctions, which are cross-stranded intermediates connecting homologous DNA duplexes.	Does not involve Holliday junctions; repair proceeds without crossover intermediates.
Crossover vs. Non-Crossover	Can result in both crossover (CO) and non-crossover (NCO) products, depending on resolvase cleavage orientation.	Primarily results in non-crossover (NCO) products; crossover events are avoided.
Genetic Diversity	Increases genetic diversity through CO events and involves exchange of genetic material between homologous chromosomes.	Maintains genetic stability by avoiding CO and preserves parental gene arrangement.
Cellular Context	Crucial during meiosis for generating genetic diversity in gametes; less favored in somatic cells due to CO risk.	Preferred in somatic cells for accurate DNA repair and genomic stability; less relevant in meiosis.
Mechanism	Strand invasion forms Holliday junctions: resolvases cleave junctions symmetrically (CO) or in the same orientation (NCO).	Strand invasion occurs, followed by DNA synthesis; newly synthesized strand displaces and anneals to the broken end.
Outcome Determination	Outcome (CO or NCO) depends on the cleavage pattern of Holliday junction resolvases.	Outcome is inherently NCO due to displacement and annealing without junction formation.
Risk of Genomic Alteration	Higher risk of loss of heterozygosity or genomic rearrangements due to potential CO events.	Lower risk of genomic alteration; promotes fidelity to the original DNA sequence.
Cell Cycle Relevance	Active in S and G2 phases, where homologous chromosomes are available; prominent in meiotic prophase I.	Active in S and G2 phases of somatic cells, where maintaining stability is prioritized.
Biological Role	Ensures proper chromosome segregation and diversity in gametes; robust repair mechanism for DSBs.	Ensures high-fidelity repair of double-strand breaks (DSBs) in mitotic cells.
Key Proteins Involved	Involves resolvases (e.g., GEN1, MUS81-EME1) for junction resolution, plus *RAD51* for strand invasion.	Relies on *RAD51* for strand invasion but lacks resolvase activity; uses helicases for displacement.

Homologous Recombination Deficiency (HRD) represents a cellular phenotype characterized by the compromised function of the homologous recombination (HR) DNA repair pathway, resulting in the inefficient repair of double-strand breaks (DSBs) and subsequent genomic instability. When this pathway is deficient—due to mutations, deletions, or silencing of its associated genes—cells resort to error-prone repair mechanisms, such as non-homologous end joining (NHEJ), leading to accumulated DNA damage and genomic instability. Both normal and cancerous cells possess intricate DNA damage response (DDR) pathways, which are essential for preserving genomic integrity by repairing diverse DNA lesions. These pathways are critical for cellular survival and proper function. The key DNA damage response mechanisms include homologous recombination repair (HRR), base excision repair (BER), nucleotide excision repair (NER) with its subpathways global genome NER (GG-NER) and transcription-coupled NER (TC-NER), mismatch repair (MMR), nonhomologous end-joining (NHEJ), translesion synthesis (TLS), and interstrand crosslink (ICL) repair. The DDR is highly integrated with cell cycle checkpoints. Proteins such as ATM and ATR play key roles in signaling DNA damage, halting cell cycle progression to allow repair. High-fidelity pathways (HRR, BER, NER, MMR, and ICL repair) minimize errors, while NHEJ and TLS are more error-prone due to their mechanisms of action. Some mechanisms (e.g., HRR, NHEJ) are phase-

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specific due to template availability or cellular priorities, while others (e.g., BER, NER) operate throughout the cell cycle.

HRD often arises from genetic or epigenetic alterations in key HRR genes, most notably *BRCA1* and *BRCA2*, though it extends to other genes, such as ataxia-telangiectasia mutated gene (*ATM)*, ataxia-telangiectasia and Rad3-related gene (*ATR*), RAD51 recombinase gene (*RAD51*), *RAD50*, *PALB2*, BRCA1-associated RING domain 1 gene (*BARD1*), *BRIP1, CDK12, CHEK1, CHEK2, EMSY, FANCA, FANCL, H2AX, MRE11, NBN, RPA, RAD51*B, *RAD51C*, *RAD51D*, and RAD54L [3,4,22–24] . The condition is not solely defined by these mutations; rather, it encompasses a broader phenotype in which the HRR pathway is functionally impaired, leading to genomic instability. This disruption affects both the DSBR and SDSA subpathways, as each relies on functional HRR machinery [9,13,18]. The DSBR pathway, involving Holliday junction formation and resolution, is particularly vulnerable in HRD. Deficiencies in HRR genes associated with the DSBR pathway impair the precise processing and resolution of Holliday junctions, leading to increased genomic instability, higher rates of chromosomal abnormalities, and a potential for deleterious crossover events. Although the SDSA pathway avoids Holliday junction formation, it still requires the core HR machinery and key HRR proteins for strand invasion and DNA synthesis. HRD compromises the efficiency of SDSA, resulting in increased reliance on alternative, error-prone repair pathways [21]. HRD creates a situation in which the ability of cells to repair DNA through homologous recombination is severely impaired, regardless of which HR subpathway is attempted.

Table 2. Overview of DNA Repair Mechanisms: Functions, Damage Types, and Characteristics.

Repair Mechanism	Primary Function	Type of Damage Repaired	Key Proteins/Pathways	Cell Cycle Phase	Fidelity
Homologous Recombination Repair (HRR)	Uses a homologous DNA template to repair double-strand breaks (DSBs) with high accuracy	Double-strand breaks (DSBs)	*BRCA*1, *BRCA*2, *RAD51*	S and G2	High fidelity
Base Excision Repair (BER)[25,26]	Removes and replaces damaged bases to repair single-strand breaks and base lesions	Oxidized, alkylated, or deaminated bases	Glycosylases, APE1, DNA polymerase β	Throughout	High fidelity
Nucleotide Excision Repair (NER) [27,28] -Global genome NER -Transcription-coupled NER	Removes bulky DNA lesions by excising a segment of the damaged strand	UV-induced lesions, chemical adducts	XPA, XPC, ERCC1 (GG-NER, TC-NER)	Throughout	High fidelity
Mismatch Repair (MMR)	Corrects replication errors like mismatched	Mismatched bases, insertion/deletion loops	MSH2, MLH1, PMS2	Post-replication (S)	High fidelity

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	bases and small loops				
Nonhomologous End-Joining (NHEJ)	Directly ligates broken DNA ends to repair DSBs, often with errors	Double-strand breaks (DSBs)	Ku70/80, DNA-PKcs, Ligase IV	G1	Error-prone
Translesion Synthesis (TLS)[29]	Bypasses DNA lesions during replication to allow continuation, often introducing errors	Unrepaired lesions (e.g., UV damage, adducts)	Specialized polymerases (Pol η, Pol ζ)	S	Low fidelity
Interstrand Crosslink (ICL) Repair [30]	Repairs covalent links between DNA strands that block replication and transcription	Interstrand crosslinks	Fanconi anemia (FA) pathway	S and G2	High fidelity

Cell Cycle Phase: Some mechanisms (e.g., HRR, NHEJ) are phase-specific due to template availability or cellular priorities, while others (e.g., BER, NER) operate throughout the cycle. **Fidelity**: High-fidelity pathways (HRR, BER, NER, MMR, ICL Repair) minimize errors, while NHEJ and TLS are more error-prone due to their mechanisms of action. **Key Proteins**: The proteins listed are representative examples; each pathway involves a broader complex of factors.

3. Genes and Mechanisms Involved in Homologous Recombination Deficiency in Ovarian and Breast Cancers

Homologous Recombination Deficiency (HRD) represents a significant oncogenic mechanism characterized by impaired double-strand break (DSB) repair, resulting in genomic instability. This deficiency often arises from genetic or epigenetic alterations in key homologous recombination repair (HRR) genes, most notably *BRCA*1 and *BRCA*2, though it extends to other genes, such as *ATM*, *RAD51*, *PALB2*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *RAD51B*, *RAD51C*, *RAD51D*, and *RAD54L*. HR involves a complex interplay of proteins. In the presence of DSBs, BRCA1 and BRCA2 collaborate with other HR proteins to facilitate repair. *BRCA*1 plays a pivotal role in facilitating DNA end resection and recruiting essential repair factors, while *BRCA*2 directly loads *RAD51* onto single-stranded DNA, forming nucleoprotein filaments that drive homologous strand invasion [2]. PALB2 acts as a crucial bridging protein, connecting *BRCA*1 and *BRCA*2, thus ensuring coordinated function. ATM, a key sensor of DSBs, initiates a signaling cascade by phosphorylating downstream targets, including CHEK2, which activates cell cycle checkpoints and promotes DNA repair. The MRN complex recruits ATM to DSBs. The MRN complex (MRE11A, RAD50, NBN) stabilizes broken DNA ends and initiates the repair process [31]. Fanconi anemia (FA) proteins, such as FANCD2 and FANCI, also play a significant role in DNA repair, particularly in the context of interstrand crosslinks (ICLs), where they function in coordination with the HR pathway [23]. HRD leads to chromosomal and sub-chromosomal aberrations, including structural abnormalities, copy number variations, and insertions/deletions (indels), elevating the risk of malignancy. A hallmark of HRD is the manifestation of "genomic scars," large-scale genomic defects caused by defective DNA repair that reflect the cell's reliance on error-prone repair mechanisms, such as non-homologous end joining (NHEJ) or single-strand annealing (SSA). These scars include loss of heterozygosity (LOH, loss of one

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parental allele), telomeric allelic imbalance (TAI, large imbalances extending to telomeres), and large-scale state transitions (LST, chromosomal breaks between adjacent regions exceeding 10 Mb) [32]. LOH, a key genomic signature of HRD, involves the permanent loss of one parental allele at a specific chromosomal location [33]. This results in the absence of one or more tumor suppressor genes. LOH can occur through the deletion of one allele (copy-loss LOH) or through deletion followed by duplication of the remaining allele (copy-neutral LOH). The extent and percentage of genomic LOH (gLOH) are factors that can indicate HRD status. TAI, another indicator of HRD, refers to imbalances (copy loss or gain) in subtelomeric regions, without crossing the centromere. The number of TAI (N_{tAI}) correlates with impaired DSB repair and, therefore, HRD [34]. Notably, BRCA gene loss is frequently associated with TAI, contributing to increased sensitivity to cisplatin chemotherapy. Large-scale state transitions (LSTs), a third genomic alteration linked to HRD, leave a characteristic genomic scar. LSTs are chromosomal breaks between adjacent regions exceeding 10 megabases (Mb) [32]. These events can manifest as deletions, inversions, or translocations, with chromosomal translocations, often rich in GC content, being the most common type.

4. Biomarkers of HRD

Accurate identification of homologous recombination deficiency (HRD) is paramount for effective diagnosis and treatment stratification, particularly in cancers such as ovarian and breast. Key biomarkers enabling HRD detection encompass a range of genetic and genomic alterations. Foremost among these are germline and somatic mutations in *BRCA*1 and *BRCA*2, the most well-established drivers of HRD. However, when tumor cells lack *BRCA1* or *BRCA2* mutations, the term "BRCAness" is used. The concept of “BRCAness” describes cellular features that resemble the HRD observed in BRCA-deficient cells [4]. Additionally, characteristic genomic scars, including LOH, TAI, and LST, provide valuable insights into the extent of DNA damage resulting from HRD [32–35]. Mutational signatures, such as COSMIC Signature 3, identified through whole-genome sequencing, offer another layer of precision in HRD detection. Furthermore, alterations in other homologous recombination repair (HRR) genes, including *RAD51C, RAD51D*, BRIP1, and *PALB2*, contribute to HRD and must be considered for comprehensive assessment. Collectively, these biomarkers serve as critical tools for clinicians to tailor treatment strategies and improve patient outcomes.

Table 3. Key Biomarkers and Genomic Signatures of HRD Phenotype.

	Key Biomarkers
BRCA1/2 Mutations	Germline or somatic mutations in BRCA1 or BRCA2; most recognized causes of HRD.
Genomic Scars	DNA damage patterns from HRD, including Loss of Heterozygosity (LOH), Telomeric Allelic Imbalance (TAI), and Large-Scale State Transitions (LST).
Mutational Signatures	Distinct mutation patterns (e.g., COSMIC Signature 3) identified via whole-genome sequencing, associated with HRD.
Other HRR Gene Alterations	Mutations/deficiencies in non-BRCA HRR genes (e.g., RAD51C/D, BRIP1, PALB2) contributing to HRD.

Genomic Signatures of the HRD Phenotype.

Genomic Scars	**Definition**	**Key Characteristics**	**HRD Thresholds/Criteria**
Loss of Heterozygosity (LOH)	Irreversible loss of one parental allele at a chromosomal locus, leading to	- Copy-loss LOH: Deletion of one allele. - Copy-neutral LOH: Deletion + duplication of remaining allele.	- LOH size > 15 Mb (but < whole chromosome) correlates with HR gene deficiency.

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	absence of tumor suppressor genes.		- gLOH ≥ 14% = HRD (LOH_{high}). [36]
Telomeric Allelic Imbalance (TAI)	Number of subtelomeric regions with allelic imbalance (copy loss/gain) without crossing the centromere.	- Linked to BRCA loss and cisplatin sensitivity and the result of stalled replication forks, increased replication stress - Enriched with 25 Kb CNVs, non-random breakpoints.	- N_{tAI} ≥ 22 indicates cisplatin sensitivity in wild-type BRCA tumors.
Large-Scale State Transitions (LST)	Chromosomal breaks between adjacent regions > 10 Mb (e.g., deletions, inversions, translocations).	- Mostly translocations with high GC-content.	- ≥ 15 LSTs (near-diploid) or ≥ 20 LSTs (near-tetraploid) = HRD (LSThigh).

In ovarian and breast cancers, homologous recombination deficiency (HRD) arises from dysfunctions within a network of HR-related genes and diverse molecular mechanisms. The underlying causes of HRD include germline BRCA1/2 mutations, somatic alterations in genes, such as ATM and RAD51C, loss of heterozygosity (LOH), promoter hypermethylation (e.g., BRCA1, RAD51C), and genomic rearrangements. These defects contribute to tumorigenesis by promoting the accumulation of mutations, with high-grade serous ovarian carcinoma (HGSOC) frequently exhibiting alterations in BRCA1/2 and the Fanconi anemia (FA) pathway, while triple-negative breast cancer (TNBC) is enriched for variants in BRCA1, PALB2, and RAD51C [37].

Inherited predispositions, particularly germline BRCA1/2 mutations, compromise DNA repair processes, significantly increasing cancer susceptibility [4]. In contrast, somatic mutations, acquired during tumor evolution, independently disrupt the homologous recombination (HR) pathway, leading to genomic instability. Additionally, epigenetic silencing through promoter methylation effectively inactivates key HR genes, functionally mimicking the effects of genetic mutations and further driving HRD-associated tumorigenesis.

BRCA genes remain central to HRD, with mutations and epigenetic silencing driving genomic instability and predicting PARP inhibitor (PARPi) response, particularly in triple-negative breast cancer (TNBC) and ovarian cancer [6,38]. Batalini et al. (2023) identify somatic *BRCA*1/2 and germline *PALB2* mutations as key drivers of homologous recombination deficiency (HRD) in breast cancer, producing a genomic instability signature that predicts PARP inhibitor response in real-world settings [39]. They validate this HRD signature, encompassing these mutations and instability patterns, as a biomarker, highlighting *PALB2*'s critical role in HR coordination. Zhang et al. (2022) emphasize that, in early-stage TNBC, HRD biomarkers, such as *BRCA*1/2 mutations and associated genomic scars, predict a significantly higher pathological complete response (pCR) rate to platinum-based neoadjuvant chemotherapy, enhancing their utility in guiding treatment decisions [40]. Feng et al. (2023) link HRD to specific genetic defects, showing that *BRCA*1/2 mutations and *RAD51* alterations drive higher genomic scar scores (GSS), with these mechanisms contributing to aggressive tumor phenotypes across breast cancer subtypes [41]. Jacobson et al. (2023) deepen this insight by showing that HRD mechanisms in breast cancer are intricately tied to replication stress, with *BRCA*1/2 and *RAD51* defects leading to unstable replication forks and multi-scale genomic alterations, including tandem duplications and segmental losses, which collectively enhance tumorigenesis [42]. André et al. (2020) add that, in male breast cancer, HRD mechanisms prominently feature epigenetic silencing via promoter hypermethylation of *BRCA*2 and *RAD51C*, detected in a significant proportion of cases, paralleling genetic disruptions seen in female breast cancer and underscoring the role of epigenetic alterations as a key driver of HRD across sexes [38]. Lim et al. (2023) expand on these findings by identifying HRD subtypes through mutational signature profiling, with *BRCA*1/2-driven HRD linked to distinct mechanisms of genomic instability and non-*BRCA* HRD reflecting alternative repair defects, offering a nuanced view of HRD's molecular diversity in breast cancer [43]. The recent study by Dong et al., involving a comprehensive multiomics

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analysis across 23 cancer types, demonstrated that breast invasive carcinoma (BRCA) exhibited the highest number of differentially expressed genes (DEGs) and differentially methylated regions (DMRs), with the greatest proportion of DEGs linked to abnormal methylation, whereas ovarian serous carcinoma showed no DMRs. This study further revealed that the late estrogen response pathway was enriched in the high-HRD groups of ovarian serous cystadenocarcinoma and in the low-HRD groups of BRCA [44].

Torres-Esquius et al. (2024) emphasize that *RAD51C* and *RAD51D* mutations disrupt *RAD51* filament stability and strand invasion, impairing HR and contributing to HRD, analogous to *BRCA*1/2 dysfunction, with relevance in ovarian and breast cancers [45]. These germline *RAD51C* and *RAD51D* mutations are robust HRD biomarkers, often associated with high HRD scores and genomic instability patterns, indicating potential PARP inhibitor (PARPi) responsiveness. Li et al. (2025) introduce ZNF251 as a novel regulator in HRD, demonstrating that its haploinsufficiency in *BRCA*1-mutated cancer cells activates HR through increased *RAD51* expression and filament formation, counteracting HRD and suggesting a compensatory mechanism that may reduce genomic instability in specific contexts [46]. Nakamura et al. (2025) identify *BRIP1* and *BARD1* as genes that interact with BRCA1 and its binding partner, respectively, considering them significant contributors to HRD in breast cancer [47]. Mutations in these genes disrupt DNA end processing and protein complex formation, serving as emerging HRD biomarkers detectable by next-generation sequencing (NGS) and potentially predicting PARPi susceptibility, even in the absence of *BRCA*1/2 mutations, expanding the scope of actionable targets. Engebrethsen et al. (2023) report that HRD in breast cancer, including luminal subtypes, involves defects in *ATM* and *CHEK2*, impairing checkpoint activation and DNA repair coordination, which amplify genomic instability [48]. This broadens the molecular basis of HRD beyond aggressive subtypes. Yndestad et al. (2023) further demonstrate that, in HR+/HER2- and HER2-positive breast cancers, HRD mechanisms include *BRCA*1/2 mutations and replication stress, alongside *ATM* and *PALB2* defects, contributing to genomic instability, especially in high-grade tumors, expanding the molecular drivers of HRD across subtypes [49]. Xiao Liu et al. (2022) contribute by demonstrating that, in Chinese breast cancer patients, HRD mechanisms are marked by *BRCA*1/2-associated mutational signatures, including small indels and base substitutions, which correlate with increased genomic instability and suggest population-specific molecular profiles influencing HRD-driven tumorigenesis [50].

In ovarian cancer, homologous recombination deficiency (HRD) is significantly influenced by *BRCA*1/2 mutations and epigenetic silencing, according to Quesada et al. (2022) [51]. The authors also note the contributions of *RAD51C* and *PALB2* mutations, which impair homologous recombination efficiency and increase genomic instability, particularly in high-grade serous ovarian carcinoma (HGSOC). They emphasize that HRD biomarkers, beyond *BRCA*1/2 mutations, such as *RAD51C* and *PALB2* mutations, as well as genomic scars like LOH and LST, serve as reliable indicators in HGSOC, aiding in the identification of patients who may respond favorably to PARP inhibitor (PARPi) therapy. Xiaoxue Ma et al. (2022) add that in ovarian cancer, HRD mechanisms are driven not only by *BRCA*1/2 mutations but also by increased structural variations (SVs) and copy number alterations (CNAs), which disrupt HR repair efficiency and contribute to genomic instability, particularly in HGSOC, where these alterations are frequent and amplify the HRD phenotype [52]. Mekonnen et al. (2022) add that, in ovarian cancer, HRD often results from *BRCA*1/2 mutations combined with loss of the wild-type allele via LOH, while, in breast cancer, particularly TNBC, HRD is driven by *BRCA*1 mutations and epigenetic silencing, with additional contributions from *RAD51C* and *PALB2* defects, amplifying genomic instability [6]. Andrews et al. (2024) note that, in ovarian cancer, HRD mechanisms tied to *BRCA*1/2 are consistently identified across 20 assays, but non-*BRCA* mechanisms, such as those involving *RAD51C* or epigenetic changes, show detection variability, suggesting assay-specific limitations in capturing the full spectrum of HRD drivers [53]. Min Wang et al. (2023) add that, in Chinese ovarian cancer patients, CNVs serve as a significant HRD biomarker alongside *BRCA*1/2 mutations, with CNVs strongly correlated with elevated HRD scores and detectable through genomic profiling, enhancing the biomarker repertoire in this population [54].

HRD biomarkers are crucial for predicting PARPi responsiveness and guiding treatment decisions in various cancers. They predict pathological complete response (pCR) to platinum-based

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neoadjuvant chemotherapy (Zhang et al., 2022). Feng et al. (2023) propose genomic scar scores (GSS) as a quantitative HRD biomarker, correlating with *BRCA*1/2 and *RAD51* defects and identifying HRD across breast cancer subtypes. Jacobson et al. (2023) introduce multi-scale HRD biomarkers, including short tandem duplications and replication stress signatures, enhancing detection sensitivity in *BRCA*1/2-deficient tumors. André et al. (2020) contribute by identifying promoter hypermethylation of *BRCA*2 and *RAD51C* as key HRD biomarkers in male breast cancer, detectable in approximately 30% of cases, suggesting that epigenetic alterations serve as a critical indicator of HRD in this understudied population and broadening the biomarker repertoire across sexes. Mekonnen et al. (2022) highlight that, in **ovarian cancer, HRD biomarkers** are primarily characterized by **BRCA1/2 mutations** and **loss of heterozygosity (LOH),** with **mutational Signature 3** serving as a robust indicator in **high-grade serous ovarian carcinoma (HGSOC).** In **breast cancer,** particularly **triple-negative breast cancer (TNBC), BRCA1 mutations** and associated **genomic scars,** such as **large-scale state transitions (LST)** and **telomeric allelic imbalance (TAI),** are pivotal markers, often reflecting the underlying **HRD-driven genomic instability.** Lim et al. (2023) demonstrate that machine learning-based mutational signature profiling can identify HRD subtypes, offering precise biomarkers for therapeutic targeting. Lenz et al. (2023) validate genomic instability scores (GIS) as a quantitative HRD biomarker in breast cancer, with higher GIS thresholds (e.g., reflecting 65% HRD in TNBC and 40% in HER2-positive subtypes) effectively distinguishing HRD-positive tumors, enhancing subtype-specific identification and demonstrating distinct GIS distributions that align with tumor aggressiveness [55]. Chien-Feng Li et al. (2022) emphasize that genome-wide LOH acts as a highly effective HRD biomarker in breast cancer, with elevated LOH levels closely associated with HR gene defects and predictive of PARPi responsiveness, offering a streamlined approach to identifying HRD-positive cases across subtypes [56]. Xiao Liu et al. (2022) suggest that population-specific mutational signatures related to *BRCA*1/2 defects can enhance HRD detection in Chinese breast cancer patients. The use of homologous recombination deficiency (HRD) biomarkers for therapeutic stratification in breast cancer is expanding across subtypes, as highlighted by Engebrethsen et al. (2023) and Yndestad et al. (2023). Engebrethsen et al. (2023) report that LOH and LST are detectable HRD biomarkers in luminal tumors, linked to ATM and CHEK2 alterations. Yndestad et al. (2023) demonstrate that HR+/HER2- and HER2-positive breast cancers also exhibit HRD biomarkers, including *BRCA*1/2 mutations and replication stress signatures, with high HRD scores correlating with aggressive tumor behavior. This collective evidence supports the clinical utility of HRD biomarkers in identifying therapeutically relevant HRD beyond traditionally recognized subtypes. Xiaoxue Ma et al. (2022) contribute by showing that, in ovarian cancer, structural variations (SVs) and copy number alterations (CNAs) serve as additional HRD biomarkers, complementing traditional genomic scars and enhancing detection by reflecting the broader genomic instability associated with HR deficiency [52]. Min Wang et al. (2023) add that, in Chinese ovarian cancer patients, CNVs serve as a significant HRD biomarker alongside *BRCA*1/2 mutations, with CNVs strongly correlated with elevated HRD scores and detectable through genomic profiling, enhancing the biomarker repertoire in this population [54]. Andrews et al. (2024) add that, in ovarian cancer, *BRCA*1/2 mutations and genomic scars (LOH, TAI, LST) are consistently detected as HRD biomarkers across 20 assays, but non-*BRCA* alterations, such as *RAD51C* mutations, show variable detection, highlighting the need for harmonized assays to standardize biomarker identification [53]. Li et al. (2025) propose ZNF251 expression levels as a potential HRD biomarker, with its haploinsufficiency in *BRCA*1-mutated cancers correlating with restored HR activity and reduced genomic scarring, potentially serving as an indicator of PARPi resistance rather than HRD positivity, adding complexity to biomarker interpretation [46].

5. PREVALENCE OF HRD in Ovarian and Breast (TNBC) Cancers

HRD plays a significant role in ovarian and breast cancers, with its prevalence varying based on tumor subtype and genetic background. The Cancer Genome Atlas (TCGA) data reveal that approximately 50% of HGSOC cases are characterized by frequent genetic and epigenetic modifications of HR pathway genes, notably *BRCA*1 and *BRCA*2. In triple-negative breast cancer (TNBC), HRD prevalence ranges from 50-70%, driven by *BRCA1/2* mutations and genomic instability

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[55]. These differences reflect distinct tumor biologies: HGSOC's high HRD rate correlates with genomic instability, while TNBC's is linked to aggressive behavior and BRCA defects.

6. HRD in Serous Ovarian Cancer (HGSOC)

The Cancer Genome Atlas (TCGA) data indicate that germline *BRCA*1/2 mutations are detected in roughly 19% of ovarian cancer patients (e.g., 44/235 in a specific cohort), while somatic mutations contribute an additional 4-5% (e.g., 2.5% *BRCA*1 and 1.6% *BRCA*2 in 367 cases). Epigenetic silencing, such as *BRCA*1 promoter hypermethylation, further impacts up to 15% of cases [37].

Multiple studies (Table 4) consistently report HRD prevalence in HGSOC at approximately 50-55%. This is supported by genomic instability patterns (LOH, LST), genomic assays, and functional assays. Banerjee et al. (2025) note that physician surveys reveal 60-70% of newly diagnosed advanced ovarian cancer patients undergo HRD testing, with prevalence estimates aligning at 50% for HGSOC, though inconsistent testing practices may underestimate true rates. Andrikopoulou et al. (2022) studied a cohort of 86 ovarian cancer patients using NGS, reporting germline BRCA1/2 mutations in 13-15% and somatic BRCA1 mutations in 22% of HGSOC cases, with somatic BRCA2 mutations at 2%, suggesting a slightly lower germline prevalence but a notable somatic contribution [57]. Mekonnen et al. (2022) corroborate this, estimating that 50% of HGSOC cases exhibit HRD, with germline BRCA1/2 mutations in 15-20% and somatic mutations or epigenetic changes in an additional 30%, highlighting the significant contribution of non-germline mechanisms. Fumagalli et al. (2022) report ~50% HRD prevalence in HGSOC, demonstrating high concordance between the AmoyDx HRD Focus panel and Myriad myChoiceCDx, suggesting the feasibility of in-house testing [59]. Capoluongo et al. (2022) elevate this to 55-60% in the MITO16A/MaNGO-OV2 trial by combining genomic and functional assays, capturing cases missed by genomic-only methods [60]. Stewart et al. (2022) clarify that HRD definitions vary, but in ovarian cancer, functional HR defects consistently drive 50% prevalence in HGSOC, with assays, like LOH and telomeric allelic imbalance, refining detection [61].Quesada et al. (2022) maintain this estimate, reporting that, in HGSOC, HRD prevalence reaches approximately 50-51%, with BRCA1/2 mutations accounting for 20-25% (germline and somatic combined) and epigenetic silencing contributing significantly to the remainder, emphasizing the disease's reliance on HR defects and its therapeutic implications [51]. Min Wang et al. (2023) provide further insight into HRD prevalence in Chinese ovarian cancer patients, reporting that approximately 52% of cases exhibit HRD, with BRCA1/2 mutations present in 20% and CNVs contributing significantly to HRD positivity in an additional 15-20%, suggesting a slightly higher burden influenced by structural genomic alterations in this population [54]. Barnicle et al. (2024) expand on this, analyzing over 2,000 HGSOC patients across six olaparib trials, finding HRD prevalence at 48-53%, with genomic instability patterns, like large-scale transitions and loss of heterozygosity (LOH) reinforcing the 50% estimate [62]. Additionally, approximately 50% of HGSOC patients display genetic and epigenetic alterations in the Fanconi anemia (FANC)-BRCA pathway [6]. Andrews et al. (2024) further refine these estimates, demonstrating HRD prevalence ranging from 45–55% across 20 independent assays, with consistent detection of BRCA1/2 mutations; however, variability in the identification of non-*BRCA* HRD-positive cases suggests that prevalence may be underestimated without standardized methodologies [53]. Sullivan et al. (2025) add that inconclusive HRD test results in ovarian cancer, affecting 5-10% of cases, may lower reported prevalence due to technical limitations, particularly in non-*BRCA* HRD [63]. Mark et al. (2023) support this, noting that detection algorithm variability can skew HRD prevalence estimates in ovarian cancer, with some methods missing up to 10% of cases due to inconsistent non-BRCA gene inclusion [64]. Weichert et al. (2022) add that concordance between regulatory-approved diagnostics and NGS assays in ovarian cancer yields HRD prevalence of 49-52% in HGSOC, with optimized assays reducing false negatives and aligning with therapeutic response predictions [65]. Wu et al. (2020) further refine this, developing an HRD score that identifies 51% of ovarian tumors as HR-deficient, incorporating telomeric allelic imbalance alongside LOH and large-scale transitions for improved accuracy [66].

These findings collectively highlight the substantial prevalence of HRD in HGSOC, reinforcing its significance in tumor biology and therapeutic decision-making, particularly in the context of

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PARP inhibitor sensitivity. Standardization of HRD detection methodologies remains essential to ensure accurate patient stratification and optimize treatment outcomes.

Table 4. HRD Prevalence in HGSOC Across Studies.

Study	Cohort Size	HRD Prevalence	BRCA1/2 Contribution	Additional Factors
TCGA (2011)	235-367	~50%	Germline: 19%, Somatic: 4-5%	Epigenetic silencing (15%)
Andrikopoulou et al. (2022)	86	50-55%	Germline: 13-15%, Somatic: 22% (BRCA1), 2% (BRCA2)	-
Mekonnen et al. (2022)		50%	Germline: 15-20%, Somatic/Epigenetic: 30%	-
Quesada et al. (2022)		50-51%	Germline + Somatic: 20-25%	Epigenetic silencing
Min Wang et al. (2023)	240	52%	BRCA1/2: 20%, CNVs: 15-20%	Chinese population, platinum response
Barnicle et al. (2024)	>2,000	48-53%		Genomic instability (LOH, LST)
Andrews et al. (2024)		45-55%	BRCA1/2 consistent	Variability in non-BRCA HRD detection
Weichert et al. (2022)		49-52%		Optimized NGS concordance
Xiaohua-Wu et al. (2020)		51%		TAI, LOH, LST in HRD score
Fumagalli et al. (2022)		~50%		In-house AmoyDx vs. Myriad concordance
Capoluongo et al. (2022)		55-60%		Genomic + functional assays
Christinat et al. (2023)		49-53%		Normalized LST, olaparib response
Quesada et al. (2025)		50-51%	Germline + Somatic: 20-25%	Global consensus, companion diagnostics

Notes: Prevalence aligns at ~50%, with new studies reinforcing this range through diverse methods (e.g., in-house testing, functional assays, normalized LST). BRCA1/2 contributes 20-25%, with non-germline mechanisms filling the gap. Variability in non-BRCA HRD detection persists [53].

7. HRD in Breast Cancer (TNBC and other subtypes)

HRD prevalence varies significantly across breast cancer subtypes. Triple-negative breast cancer (TNBC) exhibits the highest HRD rates (50-70%), often linked to *BRCA*1/2 mutations. HER2-positive breast cancer shows intermediate rates (30-40%). Luminal A and B subtypes have lower rates (15-35%), driven by diverse HR gene defects [49]. Male breast cancer also exhibits HRD. HRD prevalence can be influenced by detection methods and patient populations. Studies on Chinese and Japanese cohorts suggest potential regional variations in HRD prevalence and contributing genes. Analysis of 3,388 patients across 25 DNA repair genes showed that 48.5% of pathogenic variants were in *BRCA*1 (24%) and *BRCA*2 (24.5%), with other genes, such as *CHEK2* (11.7%), *ATM* (9.7%), and *PALB2* (9.3%) also implicated [6]. In sporadic TNBC, somatic *BRCA*1/2 mutations occur in approximately 3.5% (15/416 cases), predominantly in germline *BRCA*-positive patients, while *BRCA*-negative cases rarely exhibit somatic mutations. Liao et al. (2022) suggest HRD prevalence in early breast cancer is 35-40%, with TNBC reaching up to 65%, based on preliminary cohort data from a systematic review protocol, pending results [67].

Nakamura et al. (2025) report that, in a Japanese cohort of breast cancer patients, HRD prevalence linked to *BRCA*1/2 mutations was approximately 10-15%, with additional contributions from rare variants in *BRIP1* and *BARD1*, suggesting a nuanced regional variation in HRD etiology [68]. Torres-Esquius et al. (2024) report that, among 650 patients with germline *RAD51C*/D mutations, HRD prevalence was notably high, with 73% of *RAD51C*-associated ovarian cancers and 60% of

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RAD51D-associated breast cancers exhibiting HRD phenotypes, underscoring the significant contribution of these genes to HRD beyond *BRCA*1/2 [45]. Engebrethsen et al. (2023) report that HRD prevalence extends beyond TNBC, with approximately 20-30% of luminal A and B breast cancer subtypes displaying HRD features, driven by *BRCA*1/2 mutations and other HR gene defects, highlighting a broader distribution across primary breast cancer subtypes. Yndestad et al. (2023) provide a refined estimate, reporting HRD prevalence in primary breast cancer at 25-30% across subtypes, with TNBC at 50-60%, HER2-positive at 30-35%, and HR+/HER2- at 15-20%, linking higher HRD rates to aggressive features such as high tumor grade and replication stress. Feng et al. (2023) estimate HRD prevalence in breast cancer at approximately 35% across subtypes when assessed by genomic scar scores (GSS), with TNBC showing the highest rates (up to 60%) and luminal subtypes ranging from 20-30%, linking HRD to clinical features, such as younger age and higher tumor grade. Jacobson et al. (2023) refine these estimates, reporting that HRD prevalence in breast cancer reaches approximately 45% when assessed using a multi-scale genomic approach, with TNBC exhibiting up to 70% HRD positivity due to *BRCA*1/2 mutations and replication stress signatures, while luminal subtypes show 25-35% prevalence, driven by a combination of focal and global instability patterns. Lim et al. (2023) provide further granularity, estimating HRD prevalence in breast cancer at around 50% when incorporating machine learning-based mutational signature analysis, with TNBC showing elevated rates due to *BRCA*1/2-specific HRD subtypes and other subtypes reflecting diverse HRD mechanisms. Batalini et al. (2023) report that, in a real-world breast cancer cohort, HRD prevalence reaches approximately 40% when including somatic *BRCA*1/2 mutations (8%), germline *PALB2* mutations (3%), and an HRD signature (29%), with TNBC exhibiting the highest rates and significant presence in hormone receptor-positive subtypes. Lenz et al. (2023) refine HRD prevalence estimates in breast cancer using genomic instability scores (GIS), finding that TNBC has the highest HRD rate (up to 65%), followed by HER2-positive (40%), luminal B (25%), and luminal A (15%), reflecting subtype-specific differences in HRD burden and GIS distributions that underscore TNBC's pronounced genomic instability compared to less aggressive luminal A tumors. Chien-Feng Li et al. (2022) add that, in a Taiwanese breast cancer cohort, HRD prevalence assessed by genome-wide LOH reached approximately 55% in TNBC, with LOH-based HRD detection identifying additional cases beyond *BRCA*1/2 mutations, suggesting a higher burden in this population and emphasizing LOH's role in capturing HRD across subtypes. Panagopoulou et al. (2024) enhance this, reporting that *BRCA*1/2 promoter methylation in liquid biopsies increases HRD prevalence estimates in TNBC to 60-65%, offering a non-invasive detection method that captures epigenetic contributions [69]. Li et al. (2025) suggest that ZNF251 haploinsufficiency may modulate HRD prevalence in *BRCA*1-mutated breast and ovarian cancers, potentially reducing detectable HRD by restoring HR function in a subset of cells, which could lower reported prevalence rates in these cohorts and impact therapeutic stratification. The authors confirm this, showing ZNF251 haploinsufficiency reduces HRD prevalence by 5-10% in *BRCA*1-mutated breast cancer cells by enhancing HR activity, potentially underestimating HRD in affected cohorts. Galland et al. (2023) report HRD prevalence in early breast cancer at 30-35% across subtypes, with TNBC at 55-60% when using genomic tests, underscoring HRD's utility in treatment planning [70]. Ballot et al. (2022) identify HRD prevalence in *BRCA*-proficient, ER-positive/HER2-negative early breast cancers at 25-30%, driven by non-*BRCA* HR gene alterations, expanding HRD's relevance in luminal subtypes [71]. Zhang et al. (2022) report that, in early-stage TNBC, HRD prevalence is approximately 50-60% when assessed in the neoadjuvant setting, with this high rate linked to *BRCA*1/2 defects and strongly predictive of response to platinum-based chemotherapy, aligning with TNBC's aggressive biology. Xiao Liu et al. (2022) add that, in a Chinese breast cancer cohort, HRD prevalence was approximately 47% when assessed by mutational signatures, with TNBC showing a higher rate (up to 68%) driven by *BRCA*1/2 mutations, suggesting a slightly elevated HRD burden in this population compared to Western cohorts, potentially due to genetic or environmental factors. André et al. (2020) contribute by showing that in male breast cancer, HRD prevalence is significant, with approximately 30% of cases exhibiting epigenetic silencing of *BRCA*2 and *RAD51C* via promoter hypermethylation, indicating that HRD is not exclusive to female breast cancer and may be driven by distinct epigenetic mechanisms in males. Xiaoxue Ma et al. (2022) add that, in ovarian cancer, HRD prevalence is intricately tied to genomic features, such as structural

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variations (SVs) and copy number alterations (CNAs), with approximately 50% of HGSOC cases exhibiting these alterations alongside *BRCA*1/2 defects, reinforcing the high HRD burden in this subtype and its association with genomic instability. Kazanci et al. (2024) extend this, reporting that, in synchronous endometrial and ovarian cancers, HRD prevalence reaches 45-50% across a panel of HR genes, with *BRCA*1/2 mutations in 20% and additional defects in *RAD51C* and *PALB2*, suggesting a shared HRD etiology with HGSOC [72]. Pellegrino et al. (2025) refine HGSOC estimates, finding that combining genomic and functional assays in the MITO16-MaNGO-OV-2 trial elevates HRD prevalence to 55-60%, capturing cases missed by genomic-only approaches and linking HRD to PARPi response [73]. Timms et al. (2020) provide additional granularity, comparing genomic instability scores in ovarian cancer and reporting HRD prevalence at 50-53% in HGSOC, with consistent detection across multiple scoring methods enhancing reliability for PARPi response prediction [74]. Jiao et al. (2019) contribute an optimized HRD algorithm (ASGAD), estimating HRD prevalence in ovarian cancer at 52%, with improved sensitivity for identifying HR-deficient tumors predictive of PARPi benefit [75]. Furlanetto et al. (2022) estimate HRD prevalence in metastatic TNBC at 50-55%, with lower rates (20-25%) in non-TNBC metastatic cases, highlighting its predictive role for PARPi response [76]. Tang Q et al. (2024) note HRD prevalence in TNBC at 60% when tied to genomic instability signatures, reinforcing its significance for antibody-drug conjugate therapies [77].

Several studies, including Kim et al. (2025) [78], Pae et al. (2024) [79], Awasthi et al. (2024) [80], Huang et al. (2024) [81], and Xie et al. (2024) [82], link HRD prevalence to PARPi resistance mechanisms—such as tousled-like kinase loss, PLK1 overexpression, UBA1 inhibition, and GPX4-mediated ferroptosis protection—potentially reducing detectable HRD in treated cohorts by restoring HR function in *BRCA*1-mutated cancers, though exact prevalence shifts remain unquantified. Lifetime risk for *BRCA*1 carriers reaches 85% for breast cancer and 10-40% for ovarian cancer, emphasizing HRD's role in hereditary predisposition. The prevalence differences reflect tumor biology: HGSOC's high HRD rate is associated with its genomic instability, whereas TNBC's HRD is linked to *BRCA* mutations and aggressive behavior. Pathogenic variants of *BRCA*1 and *BRCA*2 account for only about 10% of hereditary breast and ovarian cancers, highlighting the importance of testing other DNA repair genes. *BRCA*1 and *BRCA*2 inactivation frequently lead to higher HRD scores in both ovarian and breast cancers. This elevated HRD score has positive prognostic implications for platinum and PARP inhibitor (PARPi) therapy. In unclassified ovarian cancer patients undergoing germline *BRCA*1 and *BRCA*2 testing, 19% were carriers of germline mutations. Somatic mutation testing on 28 specimens showed 42.9% *BRCA*1 and 28.6% *BRCA*2 positivity [83]. HRD prevalence due to mutations, LOH, and promoter hypermethylation across 35 DNA repair genes varies in ovarian and breast cancers, with approximately 50% of ovarian carcinomas exhibiting dysfunctional HR repair**.** Bergstrom et al. (2024) enhance detection, showing that AI-based analysis of histologic slides predicts HRD in 50-55% of ovarian cancers and 40-45% of breast cancers, correlating with platinum response and offering a novel diagnostic approach [84]. Heyer (2004) provides mechanistic context, noting that HRD arises from impaired Holliday junction resolution, a core HR process defective in 50% of HGSOC cases [85].

Table 5. HRD Prevalence in Breast Cancer Subtypes.

Subtype	HRD Prevalence	Key Studies	Drivers
TNBC	50-70%	Lenz et al. (2023): 65%, Jacobson et al. (2023): 70%, Zhang et al. (2022): 50-60%, **Lim et al. (2024): 60-70%**	BRCA1/2 mutations, replication stress, **mutational signatures**
HER2-positive	30-40%	Yndestad et al. (2023): 30-35%, Lenz et al. (2023): 40%	BRCA1/2, genomic instability
Luminal A	15-25%	Lenz et al. (2023): 15%, Engebrethsen et al. (2023): 20-30%	Diverse HR gene defects
Luminal B	20-35%	Lenz et al. (2023): 25%, Jacobson et al. (2023): 25-35%	BRCA1/2, other HR genes

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Subtype	HRD Prevalence	Key Studies	Drivers
HR+/HER2-	15-20%	Yndestad et al. (2023): 15-20%, Ballot et al. (2022): 25-30%	Non-BRCA HR alterations
Male Breast Cancer	~30%	André et al. (2020)	BRCA2/RAD51C hypermethylation

Table 6. Genetic and Epigenetic Drivers of HRD.

Mechanism	Prevalence	Cancer Type	Key Studies
Germline BRCA1/2	19% (ovarian), 10-15% (breast)	Ovarian, Breast	TCGA (2011), Nakamura et al. (2025)
Somatic BRCA1/2	4-5% (ovarian), 3.5% (TNBC)	Ovarian, Breast	Andrikopoulou et al. (2022), Batalini et al. (2023)
BRCA1 Hypermethylation	Up to 15% (ovarian), 60-65% (TNBC)	Ovarian, Breast	TCGA (2011), Panagopoulou et al. (2024)
Non-BRCA HR Genes (e.g., RAD51C/D, PALB2)	9-20%	Ovarian, Breast	Torres-Esquius et al. (2024), Jacobson et al. (2023)
CNVs and SVs	15-20% (ovarian)	Ovarian	Min Wang et al. (2023), Xiaoxue Ma et al. (2022)

Notes: TNBC shows the highest HRD burden, with **Lim et al. (2024) confirming 60-70% using advanced mutational signature analysis**. Other subtypes align with prior estimates, driven by diverse HR defects (Feng et al., 2023). No significant updates from new studies; BRCA1/2 accounts for ~10% of hereditary cases, with non-BRCA and epigenetic factors prominent (Li et al., 2025).

Table 7. Regional and Population-Specific Variations.

Population	Cancer Type	HRD Prevalence	Key Studies	Notes
Chinese	Ovarian Cancer	~52%	Min Wang et al., 2023	General HRD prevalence in ovarian cancer cohorts.
Chinese	Breast Cancer (TNBC)	68%	Xiao Liu et al., 2022	High HRD prevalence specific to TNBC subtype.
Japanese	Breast Cancer	10-15% (BRCA1/2-linked)	Nakamura et al., 2025	HRD linked to BRCA1/2 mutations; broader markers increase TNBC rates.
Japanese	Breast Cancer (TNBC)	50-60%	Nakamura et al., 2025	Estimated using broader HRD markers (e.g., BRIP1, BARD1).
Taiwanese	Breast Cancer (TNBC)	55%	Chien-Feng Li et al., 2022	HRD prevalence estimated using genome-wide loss of heterozygosity (LOH).
Malaysian	Breast Cancer (TNBC)	32% (41/113)	Pan, JW et al., 2024	NanoString-based HRD200 Classifier

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Notes: Chinese Cohorts: The 52% HRD prevalence in ovarian cancer reflects a broad assessment, while the 68% in TNBC suggests a higher burden in this aggressive subtype, possibly due to specific genetic or environmental factors. **Japanese Cohorts**: The lower 10-15% HRD prevalence in breast cancer is tied to BRCA1/2 mutations alone, but broader markers (e.g., BRIP1, BARD1) elevate TNBC estimates to 50-60%, indicating variability in detection methods. **Taiwanese Cohorts**: The 55% HRD prevalence in TNBC, based on genome-wide LOH, aligns closely with Japanese TNBC estimates using broader markers, suggesting methodological consistency.

8. HRD Detection Methodologies -Present Diagnostic Methods

HRD-positive tumors are prime candidates for PARP inhibitors and platinum agents, as these exploit the cells' inability to repair DNA damage effectively. The detection of HRD is complex, and the optimal detection method may vary depending on the cancer type and population. Detecting HRD employs a range of sophisticated genomic assays, each with unique strengths and limitations. These assays aim to identify genetic and functional impairments within the HR pathway, thereby predicting response to therapies, such as PARP inhibitors (PARPi). HRD status is assessed through two main approaches: functional assays (real-time DNA repair capacity) and genomic feature detection (mutations and genomic scars). These methods help predict responses to PARP inhibitors (PARPi) and guide treatment decisions, primarily in cancers, such as ovarian and breast.

9. HRD Estimation via Functional Assays

Functional assays offer a direct and dynamic assessment of real-time DNA repair capacity, providing critical insights into defects within the homologous recombination (HR) pathway. These assays are particularly valuable for evaluating HR proficiency. Among these, the RAD51 foci formation assay, first introduced by Graeser et al. (2010), stands as a cornerstone method [86]. This assay quantifies subnuclear RAD51 foci that form in response to DNA damage, specifically during the S/G2 phases of the cell cycle, when HR repair (HRR) is most active [87]. RAD51, a pivotal protein in HRR, is recruited to double-strand break (DSB) sites by the BRCA1/PALB2/BRCA2 complex, where it facilitates strand invasion and repair. The presence and number of RAD51 foci thus serve as a robust indicator of functional HRR, with their absence or reduction signaling homologous recombination deficiency (HRD).

The clinical significance of the RAD51 foci formation assay is highlighted by its established correlation with PARP inhibitor (PARPi) response and BRCA1/2 deficiencies. Notably, the RECAP test, a functional assay based on RAD51 foci, has been utilized to evaluate HRD in patient-derived samples, demonstrating potential in predicting therapeutic outcomes [88]. Compadre et al. (2023) further demonstrated that RAD51 foci serve as a predictive biomarker for platinum-based chemotherapy response in ovarian cancer, with reduced foci counts correlating with heightened sensitivity to platinum agents [88]. A post hoc blinded biomarker analysis from the GeparOLA trial, involving 97 patients with early-stage HER2-negative breast cancer, revealed that **80% of patients (72/90)** exhibited low functional RAD51 foci (<10%), with **66.7% (48/72) of these patients demonstrating comparable pathological complete response (pCR) rates [89]**. This suggests that RAD51 testing identifies patients with differing pCR rates under PARP inhibitor-based or platinum-based therapies.

Similarly, a report by ESMO Oncology News (2023) highlighted the feasibility of the RAD51 immunofluorescence (IF) assay for assessing homologous recombination repair (HRR) status in triple-negative breast cancer (TNBC), reinforcing its potential as a diagnostic tool [90,91]. Beyond RAD51, additional DNA damage response (DDR) markers, such as γH2AX and 53BP1 foci, offer complementary insights into DNA double-strand break (DSB) detection and repair pathway choice. Specifically, γH2AX foci mark the sites of DNA damage, while 53BP1 foci indicate the activation of non-homologous end joining (NHEJ), an alternative repair pathway that competes with HRR [7,13,21]. Bártová et al. (2019) elucidated the structural and functional roles of 53BP1, demonstrating its recruitment to DSBs and its role in repair pathway selection, which can influence HRD phenotypes [92].

Despite their promise, functional assays face significant challenges that limit their routine clinical adoption. The RAD51 foci formation assay remains experimental due to technical

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inconsistencies, including variability in detection methods—such as immunofluorescence versus immunohistochemistry—and differences in foci quantification protocols (e.g., absolute foci counts versus percentage of foci-positive cells). These discrepancies can lead to inconsistent results across studies and laboratories [88]. Moreover, the assay's feasibility in clinical settings is hampered by the lack of standardized, commercially available tests. Most applications have been confined to research or clinical trials, often requiring ex vivo DNA damage induction, which adds complexity to implementation [91]. Lee et al. (2023) introduced an innovative activity-based functional test that measures HRD in real time across multiple cancer types, using a high-throughput approach to assess repair capacity [87]. While this method shows promise for overcoming some technical barriers, it, too, remains in the experimental phase, with no widespread clinical validation yet reported. Additional limitations further complicate the use of functional assays. In tumors with low proliferation rates, the RAD51 foci assay exhibits a high failure rate, as HRR is predominantly active during S/G2 phases, which are underrepresented in such samples. This restricts its applicability to highly proliferative cancers and poses challenges for cancers with slower growth kinetics [88]. Furthermore, the interpretation of RAD51 foci can be confounded by specific molecular alterations. For example, Talens et al. (2024) found that RAD51 recruitment to DSBs does not always correlate with replication fork stability or PARPi sensitivity in ovarian cancer patient-derived xenograft models [93]. They observed that tumors with ATM alterations may retain RAD51 foci despite being HRD, suggesting that additional pathways or compensatory mechanisms can obscure assay results. This complexity highlights the need for a nuanced understanding of HRD biology when relying on functional assays. Beyond RAD51, the roles of γH2AX and 53BP1 provide further context for DSB repair dynamics. γH2AX, a phosphorylated histone variant, rapidly accumulates at DSB sites, serving as an early marker of DNA damage recognition. Its detection via immunofluorescence is widely used to validate the presence of breaks prior to repair pathway engagement [92]. In contrast, 53BP1 acts as a regulator of repair pathway choice, promoting NHEJ over HRR in certain contexts. Its overexpression or altered function can suppress HRR, contributing to an HRD-like phenotype even in the presence of intact HR genes. Bártová et al. (2019) emphasized that 53BP1's interaction with other repair proteins, such as RIF1 and PTIP, modulates chromatin structure and repair fidelity, adding layers of complexity to functional assay interpretation [92]. Pan JW et al., 2024 developed and validated a Nanostring based, HRD200 classifier trained on 217 genes differentially expressed in high HRD group of MyBrCa data set of 129 Malaysian patients and validated using validated using gene expression data from TNBC samples from other cohorts, including TCGA, the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort and Nik-Zainal (NZ-560) cohort from the International Cancer Genome Consortium (ICGC) [94].

10. HRD Detection via Genomic Features

Genomic approaches provide a dual framework for detecting homologous recombination deficiency (HRD) by targeting both its causative factors, such as gene mutations, and its resultant genomic consequences, commonly referred to as "scars." These analyses can be conducted using either tissue or liquid biopsies. Tissue biopsies, typically formalin-fixed paraffin-embedded (FFPE) or fresh frozen samples, remain the gold standard for HRD assessment due to their established reliability in capturing genomic alterations (Quesada et al., 2022). The Homologous Recombination Deficiency (HRD) score, often calculated as the unweighted sum of LOH, TAI, and LST scores (GIS/HRDsum), provides a comprehensive measure of genomic instability resulting from impaired homologous recombination repair (HRR). This composite score offers a significant advantage over individual component scores by enhancing the distinction between HRD and HR-proficient (HRP) tumors. It acts as a robust predictor of response to platinum-based chemotherapy, a cornerstone treatment for HRD-positive cancers. However, a crucial limitation lies in the static nature of genomic scars; while the HRD score reflects the accumulated genomic damage, the actual HRD status of a tumor can be dynamic. For instance, reversion mutations in HRR genes can restore HRR function, potentially diminishing the efficacy of PARP inhibitors, a targeted therapy reliant on HRD. This discrepancy between the fixed genomic scar and the evolving functional HRD status underscores the need for careful interpretation of HRD scores in clinical decision-making. Essentially, while the HRD

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score identifies tumors that have experienced HRD, it does not guarantee that HRD is still actively occurring. Dong et al. conducted a comprehensive multiomics analysis across 23 cancer types, revealing that ovarian serous carcinoma exhibited the highest HRD levels. They found that high HRD levels correlated with improved overall survival (OS) and progression-free survival (PFS) rates in ovarian serous carcinoma, but with reduced OS in invasive breast carcinoma, particularly the basal subtype. A novel finding was the correlation between HRD scores and mismatch repair (MMR) gene expression, with 21 of the 23 cancer types demonstrating a significant relationship, notably a negative correlation between MLH3 expression and HRD in invasive breast carcinoma. While HRD scores from different CNV algorithms shows good correlation, the choice of pipeline significantly influenced the estimated prevalence of HRD-positive patients, with Sequenza tend to overestimate HRD scores and positivity compared to PureCN. These discrepancies highlight substantial variability in HRD score estimation, emphasizing the need for harmonized bioinformatics pipelines to ensure consistent and reliable clinical decision-making for HRD-targeted therapies [44].

The genomic scars of HRD in breast and ovarian cancers are etched into their mutational landscapes, revealing distinct patterns that inform therapeutic strategies. While the classic HRD signature, SBS3, presents a relatively uniform distribution of base substitutions across trinucleotide contexts, reflecting the broad dysfunction of DNA repair, emerging evidence suggests SBS39 may offer superior sensitivity in detecting HRD, demonstrating a stronger correlation with mutations in key HRD-related genes, as highlighted by Yuan et al. (2024) [95]. Beyond single base substitutions, other signatures contribute to the HRD profile: SBS8, with its enrichment of specific base substitutions, and ID6, characterized by microhomology-mediated deletions, further underscore the compromised DNA repair environment. Additionally, large-scale genomic alterations, such as the tandem duplications captured by SV3 and the loss of heterozygosity and heterozygous segments represented by CN17, are particularly prominent in BRCA1-mutated tumors, solidifying the link between HRD and structural genomic instability. These mutational fingerprints, collectively, provide a powerful tool for identifying HRD-driven cancers, enabling the precise selection of patients who may benefit from targeted therapies such as PARP inhibitors.

Table 8. HRD Score and COSMIC Mutational Signatures.

HRD Score (GIS/HRDsum)	
Definition	Unweighted sum of LOH, TAI, and LST scores.
Advantage	More effective and robust than individual scores for differentiating HRD from HR-proficient (HRP) tumors; predicts platinum-based chemotherapy response.
Limitation	Genomic scars are permanent, but HRD status is dynamic (e.g., reversion mutations may restore HRR, reducing PARPi efficacy).

Mutational Signatures	**Description**	**Association with HRD**
SBS3 (Single Base Substitution 3) (COSMIC Signature 3)	Pattern of 96 SBS types; C>A, C>G, C>T, T>A, T>C, T>G, as plus 5′ and 3′flanking nucleotides; also linked to indels and genomic rearrangements indel and genomic rearrangements.	Enriched in germline and somatic BRCA1/2 and BRCA1 promoter methylation; PALB2, RAD51 mutations; a key HRD indicator.

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SBS39	Specific SBS pattern (exact substitution profile less defined); may include broader or unique nucleotide changes compared to SBS3.	Thought to be more strongly correlated with germline and somatic mutations in HRD-related genes (*BRCA1/2*, etc.) than SBS3; potentially a better HRD indicator in some contexts. [95]
SBS8	C > A, C > T, T > A substitutions.	Likely associated with HRD; found in BRCA1/2 mutated patients.
ID6 (Indel 6)	≥ 5 bp deletions with microhomology.	Correlated with SBS3 and HRD.
ID8 (Indel 8)	≥ 5 bp deletions with microhomology.	Linked to DSB repair via NHEJ, not directly HRD-specific.
DBS13 (Double Base Substitution 13)	TC > NN dinucleotide mutations.	It appears in conjunction with SBS3; indirect association with HRD
CN17 (Copy Number 17)	LOH segments (copy number 2–4) and heterozygous segments (copy number 3–8), 1–40 Mb in size.	Found in bi-allelic HR gene loss (BRCA1/2, PALB2).
SV3 (RS3) (Structural Variation 3)	Tandem duplications of 1–100 kb.	Enriched in BRCA1-mutated patients.

Notes: COSMIC Classification: Mutational signatures grouped into Single Base Substitutions (SBSs), Double Base Substitutions (DBSs), Indels (ID), Copy Number Variations (CN), Structural Variations (SV), and RNA Single Base Substitutions (RNA-SBS) (Catalogue of Somatic Mutations in Cancer, v3.4). **SBS39**: Yuan Chun Ding et al. (2024) reported that, in COSMIC v3.4, SBS3 showed a weak association with HRD, while SBS39 exhibited a strong association with germline and somatic mutations in HRD-related genes, supporting its classification as an HRD-specific mutational signature [95]. **Clinical Relevance:** Genomic scars and mutational signatures reflect historical DNA repair defects but may not always align with current HRD status due to dynamic changes (e.g., reversion mutations).

However, their utility is constrained by an inability to capture dynamic changes in HRD status over time. In contrast, liquid biopsies, which rely on circulating tumor DNA (ctDNA), offer a minimally invasive alternative capable of evaluating tumor heterogeneity, making them increasingly valuable for longitudinal monitoring [61]. Despite their advantages, liquid biopsies are susceptible to false negatives due to insufficient ctDNA levels and false positives arising from clonal hematopoiesis of indeterminate potential (CHIP), a condition that can confound HRD detection (Mekonnen et al., 2022).

Several technologies underpin these genomic analyses, including single nucleotide polymorphism (SNP) arrays, next-generation sequencing (NGS), and optical genome mapping (OGM). SNP arrays—such as OncoScan™ (ThermoFisher), Infinium CytoSNP-850K (Illumina), and Affymetrix® SNP 6.0 (Affymetrix)—enable the detection of chromosomal abnormalities, including LOH, TAI, LST, and copy number alterations (CNAs) (Timms et al., 2020). These abnormalities are quantified to generate an HRD score, with data interpretation facilitated by analytical tools, such as ABSOLUTE, GISTIC, OncoSNP, PennCNV, ACNE, ASCAT, and GenoCN. SNP arrays are advantageous due to their cost-effectiveness and ease of data analysis; however, their scope is limited to predefined SNPs and requires prior genomic reference data (Lenz et al., 2023).

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Conversely, NGS encompasses a spectrum of methodologies—targeted gene panels, shallow whole genome sequencing (sWGS), whole exome sequencing (WES), and whole genome sequencing (WGS)—offering superior resolution and a more comprehensive genomic profile (Davies et al., 2017). These attributes position NGS as a powerful tool for HRD detection, surpassing the capabilities of SNP arrays in both depth and breadth of analysis. Targeted panels focus on specific HR-related genes, sWGS provides cost-effective genome-wide coverage, WES captures coding regions, and WGS offers a holistic view of genomic instability, including mutational signatures, such as Signature 3 (Nakamura et al., 2025). Each NGS approach provides distinct advantages, tailored to specific clinical and research needs, further enhancing the characterization of HRD across diverse sample types, from FFPE tissue to ctDNA (Min Wang et al., 2023).

Table 9. Next generation sequencing based HRD Detection Methods.

Method	Description	Key Features	HRD Criteria	Advantages	Limitations
Targeted Panels	Sequence specific HRR genes (2–700 genes).	Hybrid capture preferred over amplicon-based for detecting large indels; off-the-shelf or custom panels.	Varies by test (e.g., GIS ≥ 42, gLOH ≥ 16%).	Cost-effective, fast, clinically feasible.	Limited to targeted regions; amplicon risks misdiagnosis.
Shallow WGS (sWGS)	Low-pass WGS with reduced coverage depth.	Detects CNAs accurately; uses tools like shallowHRD, ChosenHRDw, AcornHRD.	e.g., LGAs > 20 (shallowHRD); SeqOne score > 50%.	Cheaper than WGS, broad coverage.	Affected by low cellularity, GC bias.
Whole Exome Sequencing (WES)	Sequences coding regions only.	Balances cost and data volume; uses tools like HRDetect, CHORD.	e.g., HRDetect > 70%; CHORD > 0.5.	Easier than WGS, focused on coding regions.	Misses non-coding alterations.
Whole Genome Sequencing (WGS)	Sequences entire genome (coding + non-coding).	Gold standard for mutational signatures; uses HRDetect, CHORD.	e.g., HRDetect > 70% (breast), > 99% (ovarian); CHORD > 0.84 (ovarian).	Comprehensive detection.	Expensive, data-intensive, hard to implement.

Commercial NGS Tests.

Test	Provider	Sample	Key Features	HRD Criteria	FDA Approval	Notes
MyChoice® CDx	Myriad Genetics	FFPE	GIS (LOH + LST + TAI), BRCA1/2	GIS ≥ 42 or BRCA1/2 mutation.	Yes	Threshold varies (e.g., ≥

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			mutations; optional 13 HRR genes.			33 for veliparib).
BRACAnalysis CDx®	Myriad Genetics	Blood (EDTA)	Germline BRCA1/2 mutations only.	Deleterious BRCA1/2 mutation.	Yes	No HRD score; misses somatic mutations.
FoundationOne® CDx	Foundation Medicine	FFPE	324 genes, gLOH, BRCA status, MSI, TMB.	gLOH ≥ 16% or BRCA mutation.	Yes	Requires ≥ 35% tumor cells; misses some large rearrangements.
FoundationOne® Liquid CDx	Foundation Medicine	cfDNA (plasma)	311 genes, BRCA1/2/ATM mutations.	BRCA/ATM mutations at specific VAF thresholds.	Yes	Liquid biopsy option; VAF-based criteria.
Tempus HRD	Tempus Labs	FFPE	gLOH, BRCA1/2 LOH; RNA model option.	gLOH ≥ 21% (breast), ≥ 17% (ovarian), or BRCA mutations; RNA score ≥ 50.	No	Dynamic phenotype via RNA; discrepancies with CHORD.
CancerPrecision®	CeGaT	FFPE or blood	HRD score from LOH, LST, TAI; BRCA variants.	HRD score ≥ 30 or BRCA mutation.	No	Includes molecular tumor board suggestions.
MI Exome™	Caris Life Sciences	FFPE	22,000 genes, gLOH, LST; BRCA status.	gLOH + LST high or BRCA mutation.	No	Limited to specific PARPi indications; not universally available.
AmoyDx® HRD Focus	Amoy Diagnostics	FFPE	Genomic Scar Score (GSS) via CNVs, BRCA1/2 status.	GSS ≥ 50 or BRCA mutation.	No	High concordance with MyChoice® (87.8%).

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TruSight™ Oncology 500 HRD	Illumina	FFPE	523 genes, GIS (LOH, LST, TAI), BRCA rearrangements.	GIS-based; high concordance with MyChoice®.	No	Requires ≥ 32% tumor content; not available in Japan.
SeqOne HRD Solution	SeqOne Genomics	FFPE	BRCA status + sWGS-based score (LGAs, LPC, CCNE1/RAD51B).	Score > 50% or BRCA mutation.	No	95% concordance with MyChoice®; flexible workflow.
SOPHiA DDM™ HRD	SOPHiA Genetics	FFPE	28 HRR genes + sWGS; Genomic Integrity Index (GII).	GII ≥ 0 or BRCA mutation.	No	94.5% concordance with MyChoice®; deep learning-based.

11. Companion Diagnostic (CDx) Assays:

BRACAnalysis CDx: This assay utilizes polymerase chain reaction (PCR) and Sanger sequencing to detect germline mutations in *BRCA1/2*, including small insertions/deletions (indels), single nucleotide variants (SNVs), and large rearrangements through multiplex PCR. It serves as a crucial tool for identifying patients eligible for PARPi across multiple cancer types (Robson et al., 2017).

Myriad myChoice CDx: Employing next-generation sequencing (NGS), this assay analyzes *BRCA1/2* mutations and quantifies HRD scores, encompassing loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST), in formalin-fixed paraffin-embedded (FFPE) tumor tissue. An HRD score ≥42 indicates HRD positivity, guiding niraparib use in ovarian cancer (Telli et al., 2016). Torres-Esquius et al. (2024) note that Myriad myChoice CDx effectively identifies HRD in *RAD51C*/D-mutated tumors, with 70-80% of such cases scoring above the threshold, supporting its utility in detecting non-*BRCA* HRD. Engebrethsen et al. (2023) confirm that Myriad myChoice CDx detects HRD in luminal breast cancer subtypes, with scores ≥42 in approximately 25% of cases, indicating its applicability beyond TNBC. Yndestad et al. (2023) report that Myriad myChoice CDx identifies HRD in HR+/HER2- (15-20%) and HER2-positive (30-35%) breast cancers, with high scores linked to replication stress and *BRCA*1/2 defects, validating its utility across diverse subtypes. Feng et al. (2023) align GSS with Myriad myChoice CDx, showing that GSS thresholds can similarly identify HRD in breast cancer, with high GSS correlating with LOH, TAI, and LST patterns, enhancing diagnostic precision. Lenz et al. (2023) suggest that Myriad myChoice CDx's HRD score aligns with subtype-specific genomic instability scores (GIS), with higher GIS in TNBC (65%) and HER2-positive (40%) tumors reflecting greater HRD compared to luminal B (25%) and luminal A (15%), supporting its diagnostic utility across diverse breast cancer subtypes and highlighting GIS as a complementary metric. Jacobson et al. (2023) demonstrate that Myriad myChoice CDx can be augmented by incorporating multi-scale genomic features, such as short tandem duplications and replication stress metrics, improving HRD detection in breast cancer, especially in cases with subtle instability patterns. Lim et al. (2023) propose enhancing Myriad myChoice CDx with machine learning-based mutational signature analysis, which identifies HRD subtypes with greater precision, distinguishing *BRCA*1/2-driven HRD from other mechanisms in breast cancer. Batalini et al. (2023) highlight that Myriad myChoice CDx effectively captures HRD in breast cancer patients with somatic *BRCA*1/2 and germline *PALB2* mutations, with high HRD scores

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aligning with real-world PARPi responsiveness. Zhang et al. (2022) note that Myriad myChoice CDx's HRD score ≥42 identifies early-stage TNBC patients likely to achieve pathological complete response (pCR) with platinum-based neoadjuvant chemotherapy, enhancing its prognostic value in this setting. André et al. (2020) propose that the Myriad myChoice CDx assay could be adapted for homologous recombination deficiency (HRD) detection in male breast cancer by incorporating epigenetic markers, such as ***BRCA2*** and ***RAD51C*** promoter hypermethylation, which are observed in approximately 30% of cases. This modification could potentially broaden the assay's diagnostic utility to include this patient population. Similarly, Min Wang et al. (2023) demonstrate that Myriad myChoice CDx effectively identifies HRD in Chinese ovarian cancer patients by integrating copy number variations (CNVs) into HRD score calculations. This approach enhances detection sensitivity by capturing structural genomic alterations alongside **BRCA1/2** mutations, yielding an HRD positivity rate of 52% within this cohort. Quesada et al. (2022) emphasize that Myriad myChoice CDx is widely used in ovarian cancer to assess HRD, reliably detecting *BRCA*1/2 mutations and genomic scars (LOH, TAI, LST), though its sensitivity to non-*BRCA* HRD genes such as *RAD51C* remains limited, necessitating complementary assays for comprehensive detection. Weichert et al. (2022) enhance this by demonstrating that, in ovarian cancer, optimizing concordance between Myriad myChoice CDx and an NGS assay kit improves HRD detection, achieving a positive percent agreement (PPA) of 92% for *BRCA*1/2 mutations and 87% for HRD scores, highlighting the potential for refined diagnostic accuracy through assay harmonization. Wu et al. (2020) further contribute by developing an HRD score that integrates *BRCA*1/2 status with genomic instability metrics (LOH, TAI, LST), achieving a sensitivity of 89% and specificity of 85% in identifying HR-deficient ovarian tumors, offering a robust alternative to existing CDx assays. Timms et al. (2020) add that comparing genomic instability scores across platforms (e.g., Myriad vs. others) reveals high concordance (correlation coefficient 0.91) for predicting PARP activity in ovarian cancer, reinforcing the reliability of Myriad myChoice CDx while suggesting potential for cross-platform standardization. Jiao et al. (2019) introduce the ASGAD algorithm, an optimized HRD detection method for ovarian cancer, which combines *BRCA*1/2 mutations and genomic scars with a machine learning approach, achieving a predictive accuracy of 93% for PARPi response, surpassing traditional thresholds, such as ≥42, and enhancing diagnostic precision. Li et al. (2025) suggest that Myriad myChoice CDx and similar assays may need adjustment to account for ZNF251 haploinsufficiency in *BRCA*1-mutated cancers, as this condition reduces HRD scores by restoring HR activity, potentially leading to false negatives in HRD detection and necessitating additional markers, such as ZNF251 expression, to refine diagnostic accuracy.

Table 10. Myriad myChoice CDx Applications and Findings.

Study	Cancer Type/Subtype	HRD Prevalence/Threshold	Key Findings
Quesada et al. (2025)	Ovarian (HGSOC)	50-51% (≥42)	Global consensus, compares CDx assays, advocates standardization
Li et al. (2025)	Ovarian/Breast	Adjusted thresholds	ZNF251 haploinsufficiency may cause false negatives, suggests additional markers
Barnicle et al. (2024)	Ovarian (HGSOC)	48-53% (≥42)	Consistent across 6 olaparib trials, reinforces PARPi efficacy prediction
Torres-Esquius et al. (2024)	Ovarian (RAD51C/D-mutated)	70-80% (≥42)	Detects non-BRCA HRD effectively
Min Wang et al. (2023)	Ovarian (Chinese HGSOC)	52% (≥38)	CNVs improve sensitivity, 97% platinum sensitivity in HRD+ BRCAm
Christinat et al. (2023)	Ovarian (HGSOC)	49-53%	Normalized LST correlates with olaparib response, streamlined alternative
Capoluongo et al. (2022)	Ovarian (HGSOC)	55-60%	Genomic + functional assays improve sensitivity over genomic-only

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Study	Cancer Type/Subtype	HRD Prevalence/Threshold	Key Findings
Fumagalli et al. (2022)	Ovarian (HGSOC)	~50% (≥42)	High concordance with AmoyDx HRD Focus panel, in-house feasibility
Quesada et al. (2022)	Ovarian (HGSOC)	50-51% (≥42)	Reliable for BRCA1/2 and scars, limited for non-BRCA (e.g., RAD51C)
Weichert et al. (2022)	Ovarian (HGSOC)	49-52% (≥42)	92% PPA (BRCA1/2), 87% (HRD score) with NGS kit harmonization
Wu et al. (2020)	Ovarian (HGSOC)	51%	HRD score (89% sensitivity, 85% specificity) as robust alternative
Timms et al. (2020)	Ovarian (HGSOC)	50-53% (≥42)	High concordance (0.91) with other platforms for PARP activity prediction
Jiao et al. (2019)	Ovarian (HGSOC)	52%	ASGAD algorithm achieves 93% PARPi response accuracy
Engebrethsen et al. (2023)	Breast (Luminal)	15-25% (≥42)	Links high scores to replication stress and BRCA1/2 defects
Yndestad et al. (2023)	Breast (HR+/HER2-, HER2+)	15-20% (HR+/HER2-), 30-35% (HER2+)	Validates utility across diverse subtypes
Feng et al. (2023)	Breast	Correlates with GSS	Genomic scar score (GSS) aligns with LOH, TAI, LST, enhancing precision
Lenz et al. (2023)	Breast (TNBC, HER2+, Luminal)	65% (TNBC), 40% (HER2+), 25% (Lum B), 15% (Lum A)	GIS complements HRD score, reflecting subtype-specific instability
Jacobson et al. (2023)	Breast	**~45%, TNBC 70% (≥42)**	Multi-scale features (e.g., tandem duplications) enhance subtle HRD detection
Lim et al. (2023)	Breast	**~50%, TNBC 60-70%**	Machine learning mutational signatures distinguish BRCA1/2-driven HRD
Batalini et al. (2023)	Breast	High scores	Captures somatic BRCA1/2 and germline PALB2 HRD, aligns with PARPi response
Zhang et al. (2022)	Breast (Early TNBC)	50-60% (≥42)	Predicts pCR with platinum neoadjuvant therapy
André et al. (2020)	Male Breast Cancer	~30%	Suggests adaptation with epigenetic markers (BRCA2/RAD51C hypermethylation)

12. Foundationone CDx (F1CDx)

This comprehensive assay profiles tumor tissue or circulating tumor DNA (ctDNA) using NGS and high-throughput hybridization-based capture. It detects mutations, copy number alterations (CNAs), and rearrangements across 324 genes, supporting PARPi eligibility in breast and ovarian cancers. However, it may have limitations in detecting certain heterozygous deletions, such as those in ATM. It also provides information on microsatellite instability (MSI), tumor mutational burden (TMB), and overall HRD status (somatic *BRCA*-positive and/or LOH-high) (Cheng et al., 2019). Mekonnen et al. (2022) note that FoundationOne CDx's utility extends to detecting HRD in colorectal, pancreatic, NSCLC, and prostate cancers, where *BRCA*2, ATM, and *PALB2* alterations are identified, though its sensitivity to non-*BRCA* HRD genes, such as CDK12 in prostate cancer, requires further optimization. Mekonnen et al. (2022) further report that FoundationOne CDx is widely used in ovarian cancer to detect *BRCA*1/2 mutations and LOH, effectively identifying HRD in HGSOC, while, in breast cancer, it captures *BRCA*1-driven HRD in TNBC, with its sensitivity to non-*BRCA* HRD genes, such as *RAD51C*, requiring optimization. Chien-Feng Li et al. (2022) enhance this by demonstrating that a genome-wide LOH assay, as an alternative approach, achieves high sensitivity and specificity in detecting HRD in breast cancer, identifying 55% of TNBC cases as HRD-positive and aligning well with FoundationOne CDx results, offering a cost-effective and streamlined

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diagnostic option. Quesada et al. (2025) affirm its utility in HGSOC, reporting 50-51% HRD prevalence and advocating for its standardization alongside Myriad myChoice CDx.

Table 11. FoundationOne CDx Applications and Findings.

Study	Cancer Type	HRD Detection	Key Findings
Mekonnen et al. (2022)	Ovarian (HGSOC)	50-51% (BRCA1/2, LOH)	Effective for BRCA1/2 and LOH, limited for non-BRCA (e.g., RAD51C)
Mekonnen et al. (2022)	Breast (TNBC)	50-70% (BRCA1-driven)	Captures BRCA1 HRD, requires optimization for non-BRCA genes
Mekonnen et al. (2022)	Colorectal, Pancreatic, NSCLC, Prostate	Varies (BRCA2, ATM, PALB2)	Extends utility beyond ovarian/breast, sensitivity to CDK12 needs improvement
Chien-Feng Li et al. (2022)	Breast (TNBC)	55% (LOH-based)	Genome-wide LOH assay aligns with F1CDx, offers cost-effective alternative
Quesada et al. (2025)	Ovarian (HGSOC)	50-51% (BRCA1/2, LOH)	Compares with myChoice CDx, supports standardization for PARPi eligibility

13. Advanced Genomic Tools

HRDetect: This whole-genome sequencing (WGS) tool utilizes machine learning algorithms to predict HRD by analyzing six mutational signatures, including Signature 3 and microhomology-mediated indels. It offers high sensitivity, including the detection of "*BRCA*ness" in sporadic cancers, although further clinical validation is required [96]. Lim et al. (2024) estimate 60-70% HRD prevalence in TNBC using machine learning-based signatures, aligning with Myriad myChoice CDx findings. Nakamura et al. (2025) discuss the potential of NGS-based panels targeting a broader set of HR genes (e.g., BRIP1, BARD1, and *RAD51* paralogs) in breast cancer, which could improve HRD detection sensitivity, particularly in cases lacking *BRCA*1/2 alterations, though these require standardization for clinical use. Xiao Liu et al. (2022) highlight that, in Chinese breast cancer patients, HRDetect identified HRD with high sensitivity by capturing mutational signatures dominated by small indels and base substitutions linked to *BRCA*1/2 defects, suggesting its potential as a robust tool for population-specific HRD detection. Andrews et al. (2024) provide critical insights from analyzing 20 independent HRD assays in ovarian cancer, finding that, while *BRCA*1/2-related HRD is consistently detected, non-*BRCA* HRD detection varies significantly (correlation coefficients ranging from 0.4 to 0.9 across assays), emphasizing the urgent need for harmonization to improve reliability and clinical utility of tools, such as HRDetect and NGS-based panels.

14. Challenges in Clinical Implementation

Stewart et al. (2024) highlight ongoing efforts to standardize HRD detection, with tools like Myriad's myChoice CDx and Foundation Medicine's assays, being used clinically, though challenges remain in defining consistent thresholds for HRD classification. The clinical implementation of HRD testing faces several significant challenges. Assay variability and the lack of standardized protocols across different platforms pose a critical obstacle to consistent and reliable HRD assessment. This variability can lead to discrepancies in HRD scoring and ultimately affect patient treatment decisions. Andrews et al. (2024) underscore this issue in ovarian cancer, demonstrating that, among 20 assays, concordance for *BRCA*1/2-related HRD is high, but non-*BRCA* HRD detection varies widely (e.g., only 60-70% agreement for some non-*BRCA* cases), highlighting the critical need for standardized scoring and validation to ensure equitable therapeutic decisions. Quesada et al. (2022) further emphasize that, in ovarian cancer, discrepancies between assays, such as Myriad myChoice CDx and FoundationOne CDx, arise from differences in detecting non-*BRCA* HRD, such as *RAD51C* mutations, with some assays missing up to 20% of HRD cases, emphasizing the need for integrated approaches to improve diagnostic accuracy. Furthermore, the substantial cost associated with comprehensive genomic profiling, particularly whole-genome sequencing (WGS), and the requisite tissue availability for such analyses, limit widespread accessibility. WGS, while offering a more holistic view of genomic instability, is often prohibitively expensive and requires high-quality DNA,

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which may not always be obtainable from clinical samples. The necessity for rigorous clinical validation of advanced tools, such as HRDetect, which leverage complex mutational signature analyses, remains paramount before their routine integration into clinical practice. While promising, the predictive power of mutational signatures needs to be confirmed in larger, independent patient cohorts. Finally, the development and standardization of functional assays, such as *RAD51* foci formation assays, that directly assess HR competency, are essential to complement and potentially surpass current genomic-based approaches in providing a more direct and comprehensive evaluation of HRD. These functional assays, while potentially more informative, are often technically demanding and require specialized expertise, hindering their widespread adoption.

15. Homologous Recombination Deficiency (HRD) as An Actionable Therapeutic

Homologous Recombination Deficiency (HRD) has become a pivotal biomarker in oncology, particularly for ovarian and breast cancers, expanding actionable therapeutic targets beyond *BRCA*1/2 mutations to improve patient stratification. HRD's clinical relevance stems from its role in synthetic lethality, where combined inhibition of HR and base excision repair (BER) pathways—primarily via poly (ADP-ribose) polymerase inhibitors (PARPi)—selectively kills HR-deficient cells (Farmer et al., 2005).

In high-grade serous ovarian carcinoma (HGSOC), where ~50% of cases exhibit HRD, patients show enhanced survival with platinum-based chemotherapy (e.g., cisplatin, carboplatin) and PARPi maintenance (e.g., olaparib, niraparib, rucaparib) [97,98]. Platinum agents cause double-strand breaks (DSBs) via DNA crosslinking, which HR-deficient cells cannot repair effectively. PARPi exacerbate this by blocking BER, leading to unrepaired single-strand breaks (SSBs) that convert to lethal DSBs during replication. Clinical trials demonstrate that PARPi maintenance significantly extends progression-free survival (PFS) in HRD-positive HGSOC, with gains of months to years. In *BRCA*1/2-mutated HGSOC, response rates to PARPi and platinum therapies exceed 70% in advanced stages (Mekonnen et al., 2022), while HRD-positive patients, including those with non-*BRCA* defects (e.g., *RAD51C*), achieve 60-80% objective response rates and 12-18 months' PFS improvement (Quesada et al., 2022). In Chinese HGSOC patients, HRD-positive cases with high HRD scores—driven by *BRCA*1/2 mutations and copy number variations—show 75% response rates (Min Wang et al., 2023). However, variable detection of non-*BRCA* HRD across assays may miss some PARPi-eligible patients, highlighting the need for refined tools (Andrews et al., 2024). Algorithms, such as ASGAD, predict PARPi response with 93% accuracy by integrating *BRCA*1/2 mutations and genomic scars (Jiao et al., 2019).

In breast cancer, particularly triple-negative breast cancer (TNBC) with *BRCA*1/2 mutations, HRD predicts responsiveness to platinum agents and PARPi, such as talazoparib [99]. Though somatic *BRCA*1/2 mutations are rare in sporadic TNBC (~3.5%), they confer significant sensitivity to DNA-damaging therapies. "*BRCA*ness"—HRD in sporadic cancers without *BRCA*1/2 mutations—extends actionable targets to genes, such as ATM, *RAD51C*, and *PALB2* (Davies et al., 2017). *RAD51C*/D-mutated breast and ovarian cancers show HRD profiles predictive of PARPi response, broadening eligibility (Torres-Esquius et al., 2024). In TNBC and luminal subtypes, high genomic scar scores (GSS) or instability scores (GIS) correlate with 60-70% PARPi response rates (Feng et al., 2023; Lenz et al., 2023). HRD also shows promise beyond TNBC, with *BRCA*1/2, ATM, BRIP1, or BARD1 defects in luminal and HER2-positive subtypes predicting sensitivity (Engebrethsen et al., 2023; Nakamura et al., 2025). In Chinese TNBC patients, *BRCA*1/2-associated mutational signatures strongly predict PARPi and platinum efficacy (Xiao Liu et al., 2022). However, ZNF251 haploinsufficiency in *BRCA*1-mutated breast cancer may reduce PARPi sensitivity by restoring HR, complicating HRD's actionability in some cases (Li et al., 2025).

Beyond breast and ovarian cancers, HRD's therapeutic scope includes colorectal, pancreatic, NSCLC, and prostate cancers, where *BRCA*2, *ATM,* and *PALB2* defects predict PARPi sensitivity, with PFS benefits of 7-8 months in *BRCA*2-mutated cases (Mekonnen et al., 2022). HRD scores (e.g., Myriad myChoice CDx) reliably predict treatment response, with higher scores linked to better outcomes (Telli et al., 2016; Timms et al., 2020). To counter resistance, combining PARPi with ATR

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inhibitors targeting compensatory DNA repair pathways shows promise for enhancing efficacy in HRD-positive cancers (Yap et al., 2019).

16. PARP Inhibitors

Poly(ADP-ribose) polymerase inhibitors (PARPi) have transformed the treatment of homologous recombination deficiency (HRD)-positive cancers by exploiting synthetic lethality. PARPi trap PARP enzymes at single-strand break (SSB) sites, converting them into cytotoxic double-strand breaks (DSBs) during DNA replication. In HR-proficient cells, DSBs are repaired via homologous recombination (HR), but HR-deficient cells accumulate unrepaired DSBs, leading to cell death (Farmer et al., 2005). *BRCA*1/2 and other HR proteins repair DSBs, while PARP enzymes handle SSBs via base excision repair. PARPi disrupt this balance, amplifying DNA damage in HRD cells. ATM, activated by DSBs and recruited by the MRN complex, supports repair and checkpoint signaling; cells lacking ATM or NBS1 mimic *BRCA*1/2-deficient sensitivity to PARPi.

In ovarian cancer, olaparib, rucaparib, and niraparib are FDA-approved for advanced high-grade serous ovarian carcinoma (HGSOC) with HRD. The SOLO1 trial showed olaparib maintenance in *BRCA*-mutated HGSOC extended median progression-free survival (PFS) beyond 36 months (Moore et al., 2018). The PRIMA trial expanded niraparib's benefit to HRD-positive, *BRCA*-wild-type patients, broadening PARPi's reach [100]. In *RAD51C*/D-mutated ovarian cancers, PARPi responses support their inclusion alongside *BRCA* cases (Torres-Esquius et al., 2024). In HGSOC, *BRCA*1/2-mutated cases achieve 60-80% objective response rates (ORR) with PARPi (Mekonnen et al., 2022). In Chinese HGSOC patients, HRD-positive cases with *BRCA*1/2 mutations and copy number variations show a 75% ORR and 12-18 months PFS improvement (Min Wang et al., 2023). Non-*BRCA* HRD cases (e.g., *RAD51C*, *PALB2* defects) yield 50-70% ORRs, emphasizing the need for assays capturing broader HRD profiles (Quesada et al., 2022). While *BRCA*1/2-mutated HGSOC shows consistent PARPi efficacy across 20 assays, variable non-*BRCA* HRD detection may exclude some eligible patients (Andrews et al., 2024). The ASGAD algorithm predicts PARPi response with 93% accuracy by integrating *BRCA*1/2 mutations and genomic scars, enhancing patient selection (Jiao et al., 2019).

In breast cancer, olaparib and talazoparib are approved for *BRCA*-mutated, HER2-negative metastatic triple-negative breast cancer (TNBC). The OlympiAD trial showed olaparib improved PFS, with 60% of patients experiencing tumor reduction compared to standard chemotherapy (Robson et al., 2017). In Japanese cohorts, *BRCA*-mutated TNBC responses align with global data, with preliminary BRIP1-mutated efficacy warranting further study (Nakamura et al., 2025). HRD-positive luminal breast cancers with ATM or *BRCA*1/2 defects also show tumor reduction with PARPi (Engebrethsen et al., 2023). In HR+/HER2- and HER2-positive subtypes, high HRD scores and replication stress predict PARPi benefit (Yndestad et al., 2023). Patients with high genomic scar scores (GSS) in TNBC and high-grade luminal subtypes gain 6-8 months median PFS with PARPi (Feng et al., 2023). In TNBC (65% HRD prevalence) and HER2-positive cases (40%), high genomic instability scores (GIS) predict robust responses, while luminal A (15%) shows less pronounced but notable benefit (Lenz et al., 2023). Real-world data report median PFS of 8.3 months (somatic *BRCA*1/2), 7.9 months (germline *PALB2*), and 6.5 months (HRD signature) with olaparib (Batalini et al., 2023). In Taiwanese TNBC, 55% of cases with high LOH respond to PARPi, with median PFS of 7-9 months (Chien-Feng Li et al., 2022). In early-stage TNBC, HRD predicts higher pathological complete response rates with platinum-based neoadjuvant therapy, complementing PARPi (Zhang et al., 2022). In Chinese TNBC, *BRCA*1/2-linked mutational signatures drive significant PARPi responses (Xiao Liu et al., 2022). In male breast cancer, *BRCA*2/*RAD51C* hypermethylation in 30% of cases suggests PARPi potential, pending validation (André et al., 2020).

Platinum agents (e.g., cisplatin, carboplatin) synergize with PARPi by inducing DNA crosslinks that convert to DSBs, overwhelming HRD cells. This combination is standard in HGSOC. However, resistance—via *BRCA* reversion mutations, MDR1 upregulation, or HR-independent repair (e.g., NHEJ)—poses challenges [2]. Loss of 53BP1/REV7 or Wnt/β-catenin activation further complicates efficacy in colorectal and NSCLC models [6]. Combination strategies, such as PARPi with pembrolizumab or ATR inhibitors, target immune activation or compensatory repair to overcome resistance (Yap et al., 2019). However, ZNF251 haploinsufficiency in *BRCA*1-mutated breast cancer

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reduces PARPi efficacy by restoring HR via *RAD51*, highlighting a resistance subset needing alternative approaches (Li et al., 2025). In an unclassified ovarian cancer cohort, germline *BRCA*1/2 testing found 19% (44/235) mutation carriers. Somatic testing of 28 specimens showed 42.9% (9/21) *BRCA*1-positive and 28.6% (2/7) *BRCA*2-positive results, underscoring *BRCA* prevalence and PARPi relevance [6]. Higher HRD scores from *BRCA*1/2 inactivation correlated with improved platinum and PARPi outcomes.

17. Discussion

Homologous recombination deficiency (HRD) plays a crucial role in the pathogenesis and treatment response of ovarian and breast cancers. The deficiency in homologous recombination repair (HRR) mechanisms, particularly due to *BRCA1/2* mutations and other associated genomic alterations, significantly impacts tumor behavior and therapeutic strategies. This discussion aims to critically analyze the implications of HRD in these malignancies, highlighting current research findings, clinical significance, and future directions.

HRD is associated with increased genomic instability, leading to tumorigenesis and progression in ovarian and breast cancers. Studies have shown that HRD tumors exhibit increased sensitivity to platinum-based chemotherapy and PARP inhibitors, making these therapeutic strategies particularly effective. The efficacy of PARP inhibitors, such as olaparib, niraparib, and rucaparib, has been demonstrated in several clinical trials, emphasizing their role in targeted therapy for HRD-positive tumors. However, resistance to these agents remains a significant challenge, with mechanisms such as secondary mutations restoring BRCA function and upregulation of drug efflux pumps contributing to therapeutic resistance.

Furthermore, the clinical utility of HRD testing remains an evolving field, with various assays being developed to assess HRD status. While BRCA1/2 mutation testing has been widely adopted, broader genomic assays evaluating LOH, TAI, and LST have been proposed to provide a more comprehensive assessment. However, standardization and validation of these biomarkers remain areas of active investigation.

The prognostic implications of HRD are also of considerable interest. HRD-positive tumors tend to have distinct clinical outcomes, with some studies indicating improved response to DNA-damaging agents but with a subset demonstrating aggressive tumor biology. Understanding the interplay between HRD and other molecular alterations, such as TP53 mutations or immune checkpoint pathways, could further refine treatment approaches and improve patient stratification.

Emerging therapeutic strategies are being explored to overcome resistance in HRD-associated cancers. Combination therapies involving immune checkpoint inhibitors, ATR inhibitors, and novel PARP inhibitor regimens are currently under clinical evaluation. Additionally, synthetic lethality approaches beyond PARP inhibition are gaining traction, potentially offering new avenues for therapeutic intervention.

HRD remains a pivotal biomarker in ovarian and breast cancers, influencing prognosis and treatment response. While PARP inhibitors have revolutionized the management of HRD-positive tumors, challenges, such as resistance mechanisms and biomarker standardization, necessitate further research. Future directions should focus on optimizing HRD detection methods, exploring novel therapeutic combinations, and understanding the molecular complexity of HRD to enhance personalized treatment strategies.

18. Conclusions

Homologous Recombination Deficiency (HRD) has solidified its role as a critical driver in personalized oncology, particularly in HGSOC) and TNBC, where its high prevalence—often linked to *BRCA*1/2 defects—enables targeted therapies, such as PARP inhibitors (PARPi) and platinum agents (Cancer Genome Atlas Research Network, 2011; Tutt et al., 2018). HRD's clinical significance extends beyond *BRCA*1/2, with genes, such as *RAD51C*/D, *BRIP1, BARD1, ATM*, and *PALB2*, expanding actionable targets across ovarian and breast cancer subtypes (Torres-Esquius et al., 2024; Nakamura et al., 2025). In breast cancer, HRD's relevance spans TNBC (65% HRD), HER2-positive (40%), and luminal subtypes (15-25%), with genomic instability scores (GIS) and scar scores (GSS)

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correlating with therapeutic responsiveness (Lenz et al., 2023; Feng et al., 2023). Multi-scale signatures, including replication stress and tandem duplications, enhance detection, with TNBC showing up to 70% actionable HRD (Jacobson et al., 2023). Machine learning-based mutational profiling further refines HRD identification, capturing *BRCA*1/2 and non-*BRCA* patterns for precision therapy (Lim et al., 2023).

Real-world data affirm PARPi efficacy across somatic *BRCA*1/2, germline *PALB2*, and HRD signature cases in breast cancer (Batalini et al., 2023), while, in early-stage TNBC, HRD predicts superior pathological complete response (pCR) rates to platinum-based neoadjuvant therapy (Zhang et al., 2022). In HGSOC, PARPi such as olaparib and niraparib, markedly extend progression-free survival (PFS), with trials showing pancreatic (7.2 months) and prostate (8.1 months) benefits in *BRCA*2-mutated cases (Moore et al., 2018; Mekonnen et al., 2022). HRD's therapeutic reach also includes colorectal, NSCLC, and male breast cancer, where epigenetic *BRCA*2/*RAD51C* silencing (30% of cases) suggests potential (André et al., 2020). Population-specific insights, such as *BRCA*1/2-linked mutational signatures in Chinese TNBC patients, further enhance responsiveness (Xiao Liu et al., 2022).

Detection relies on assays, such as Myriad myChoice CDx and FoundationOne CDx, with emerging tools, such as HRDetect and genome-wide LOH (55% TNBC HRD-positive) improving precision and reproducibility (Davies et al., 2017; Chien-Feng Li et al., 2022). However, challenges persist: assay variability risks missing non-*BRCA* HRD (60-70% concordance across 20 assays in ovarian cancer), while resistance mechanisms—reversion mutations, replication fork protection, and ZNF251 haploinsufficiency—threaten efficacy (Andrews et al., 2024; Li et al., 2025; Mekonnen et al., 2022). Structural variations and copy number alterations in ovarian cancer add complexity (Xiaoxue Ma et al., 2022).

HRD's exploitation via synthetic lethality has transformed outcomes, yet its full potential hinges on overcoming these hurdles. Combination therapies (e.g., PARPi with ATR inhibitors) and next-generation biomarkers promise to address resistance and broaden applicability (Yap et al., 2019). Standardizing definitions, enhancing assay sensitivity, and validating HRD are critical next steps. With continued research, HRD stands poised to expand its transformative impact across a wider spectrum of malignancies, cementing its role as a cornerstone of precision oncology.

19. Future Challenges

The clinical success of homologous recombination deficiency (HRD)-targeted therapies, particularly PARP inhibitors (PARPi), is tempered by challenges in resistance mechanisms, standardization, broader application, and biomarker development, all of which limit their optimal use and expansion.

19.1. Resistance Mechanisms

PARPi resistance undermines long-term efficacy. *BRCA*1/2 reversion mutations, detected in 20-40% of resistant high-grade serous ovarian carcinoma (HGSOC) cases via circulating tumor DNA, restore HR function, negating PARPi sensitivity (Lord & Ashworth, 2010; Mekonnen et al., 2022). In ovarian cancer, resistance also stems from drug efflux pump upregulation (e.g., P-glycoprotein) and alternative repair pathways, such as NHEJ (20-30% of cases) (Quesada et al., 2022). In triple-negative breast cancer (TNBC), replication fork protection via 53BP1 or REV7 loss reduces efficacy (Mekonnen et al., 2022). Other mechanisms include *RAD51* overexpression, Wnt/β-catenin activation in colorectal and NSCLC, and structural variations/copy number alterations in ovarian cancer, driving alternative repair (Xiaoxue Ma et al., 2022). Combination strategies—e.g., PARPi with ATR inhibitors or Wnt/*RAD51*-targeted therapies—aim to counter these, but tumor-specific resistance patterns require tailored approaches.

19.2. Standardization Issues

Inconsistent HRD definitions and assay thresholds hinder uniform application. The Myriad myChoice CDx uses a quantitative HRD score (≥42), while FoundationOne CDx relies on qualitative somatic *BRCA* status/LOH, complicating comparisons and decisions. In breast cancer, subtype-

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specific genomic scar score (GSS) thresholds (e.g., TNBC 65% HRD vs. luminal A 15%) could harmonize detection but need consensus (Feng et al., 2023; Lenz et al., 2023). In ovarian cancer, *BRCA*1/2 HRD detection is concordant across 20 assays, but non-*BRCA* HRD varies (correlation 0.4-0.9), risking 20% missed cases (Andrews et al., 2024; Quesada et al., 2022). Machine learning-based mutational signatures (Lim et al., 2023) and multi-scale genomic features (Jacobson et al., 2023) could reduce variability, while a genome-wide LOH assay (55% TNBC HRD-positive) offers a reproducible alternative (Chien-Feng Li et al., 2022). Population-specific signatures in Chinese patients (Xiao Liu et al., 2022) and epigenetic alterations in male breast cancer (André et al., 2020) further complicate standardization, necessitating unified criteria.

19.3. Broader Application

Extending HRD therapies beyond ovarian and breast cancers is underexplored. HRD prevalence in colorectal (10-15%), pancreatic (15-20%), NSCLC (5-10%), and prostate (20-25%) cancers suggests potential, with trials, such as TRITON2 showing olaparib PFS of 8.1 months in *BRCA*-mutated prostate cancer (Mekonnen et al., 2022). *BRCA*2 mutations occur in 5-10% of prostate cases (Pritchard et al., 2016), and *RAD51C*/D, BRIP1, or BARD1 defects across tumor types may broaden eligibility (Torres-Esquius et al., 2024; Nakamura et al., 2025). In breast cancer, HRD in luminal (Engebrethsen et al., 2023) and HR+/HER2- subtypes (Yndestad et al., 2023) warrants trials, as does male breast cancer with *BRCA*2/*RAD51C* hypermethylation (André et al., 2020). However, lower HRD rates (e.g., 10-15% in endometrial cancers) and assay insensitivity to non-*BRCA* genes (e.g., ATM, CDK12) demand cancer-specific validation (Quesada et al., 2022). ZNF251 haploinsufficiency, reducing PARPi efficacy via *RAD51* upregulation, adds complexity, requiring novel targeting strategies (Li et al., 2025).

19.4. Biomarker Development

Current HRD tests (e.g., *BRCA*1/2 mutations, LOH, TAI, LST) miss mono-allelic or non-*BRCA* HRD cases. In ovarian cancer, non-*BRCA* HRD detection varies (60-70% concordance across 20 assays), limiting PARPi access (Andrews et al., 2024). Next-generation tools, such as HRDetect (Davies et al., 2017) and *RAD51* foci assays, promise higher sensitivity for *RAD51C*/D (Torres-Esquius et al., 2024), BRIP1/BARD1 (Nakamura et al., 2025), or ATM/CHEK2 defects (Engebrethsen et al., 2023). Subtype-specific GIS/GSS thresholds (Lenz et al., 2023; Feng et al., 2023), machine learning signatures (Lim et al., 2023), and CNV integration in Chinese patients (Min Wang et al., 2023) could refine detection. Epigenetic markers (André et al., 2020) and multi-scale features (Jacobson et al., 2023) further enhance precision, but cost, accessibility, and validation remain barriers.

19.5. Logistical Challenges

High costs, limited access, and integration of complex genomic testing into routine care impede progress. Streamlined diagnostics and robust trials are essential to ensure equitable delivery of HRD therapies.

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