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# Homologous recombination repair deficiency (HRD): From biology to clinical exploitation

David Gonzalez[1] | Albrecht Stenzinger[2,3,4]

[1]Patrick G Johnston Centre for Cancer Research, Queens University Belfast, Belfast, UK

[2]Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany

[3]German Cancer Consortium (DKTK), Heidelberg, Germany

[4]German Center for Lung Research (DZL), Heidelberg, Germany

**Correspondence**
Albrecht Stenzinger, Institute of Pathology, University Hospital Heidelberg, Im Neuenheimer Feld 224, 69120 Heidelberg, Germany.
Email: albrecht.stenzinger@med.uni-heidelberg.de

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Significant advances in cancer precision medicine over the last decade have led to a number of treatment options that have significantly improved patient outcomes. The underlying concept integrates detailed insights into disease mechanisms with clinical management where the understanding of specific vulnerabilities can be exploited for the development of drugs and therapies. One exciting aspect in this context is the rapidly evolving field of drugs that exploit defects in different DNA repair mechanisms. A recent major success is undoubtedly the high efficacy of checkpoint inhibition in tumors with high microsatellite instability (MSI-H).[1] Another evolving area is centered around the concept of homologous recombination repair deficiency (HRD), which, similar to MSI-H cases, appears to occur across cancer types at different frequencies.[2] The special issue "*Homologous Recombination Repair Deficiency (HRD): from Biology to Clinical exploitation*" highlights recent progress in the field and provides an overview on scientific and clinical developments.

Over the last years, evidence that many cancer types exhibit defects within the homologous recombination repair (HRR) machinery has accumulated. HRR, a conservative mechanism predominantly acting in S and G2 phases of the cell cycle, restores the original DNA sequence at a site where double strand breaks occur. Impairment of this machinery, or HRD, which occurs at variable frequencies across cancer types,[2] is caused by a loss of function in HRR mediators. Biologically, the impairment of the HRR pathway forces cells to utilize other mechanisms of DNA repair such as non-homologous end joining,[3] which is comparably more error prone. Based on the concept of synthetic lethality,[4] this biological phenomenon can be exploited therapeutically since a tumor exhibiting HRD is associated with a specific ("BRCAness") phenotype characterized by sensitivity to platinum-based therapies and PARP-inhibition.[5] The most common currently known causes of HRD are loss of function mutations in *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *PALB2* and a few other genes[6] as well as promoter hypermethylation of *BRCA1*.[7] However, since the number of genes involved in HRR is high, their individual biological impact is diverse and interaction is complex, we are currently far from understanding the biological impact, let alone the clinical implications, of individual (germline or somatic, mono- vs bi-allelic) aberrations in many of the other HRR-coding genes. This scenario warrants further research efforts including tools, such as the one developed by Kolb and colleagues,[8] that can be used to investigate HRD scenarios in preclinical models. It also poses a major challenge to the interpretation of clinical trial data where non BRCA1/2-HRR genes mutated at low frequencies and often identified by a tumor-sequencing only approach (which is unable to differentiate between somatic and germline events) are often lumped and analyzed in groups, which reflect only to a very limited extent the underlying biology and complexity of the network.[9-11] The work by Hirsch et al.[12] and George and Turnbull[13] provides guidance on the interpretation and classification of mutations in individual HRR genes, which are nowadays often identified by large NGS gene panels (≥1 MB) used in precision oncology programs. In this context, it is important to keep in mind that clinical trials investigating the efficacy of PARP inhibitors adopted a variant classification system for response prediction that was originally developed for risk prediction of germline carriers and their relatives.[14,15] This approach is also used in routine diagnostics when looking at variants in individual HRR genes

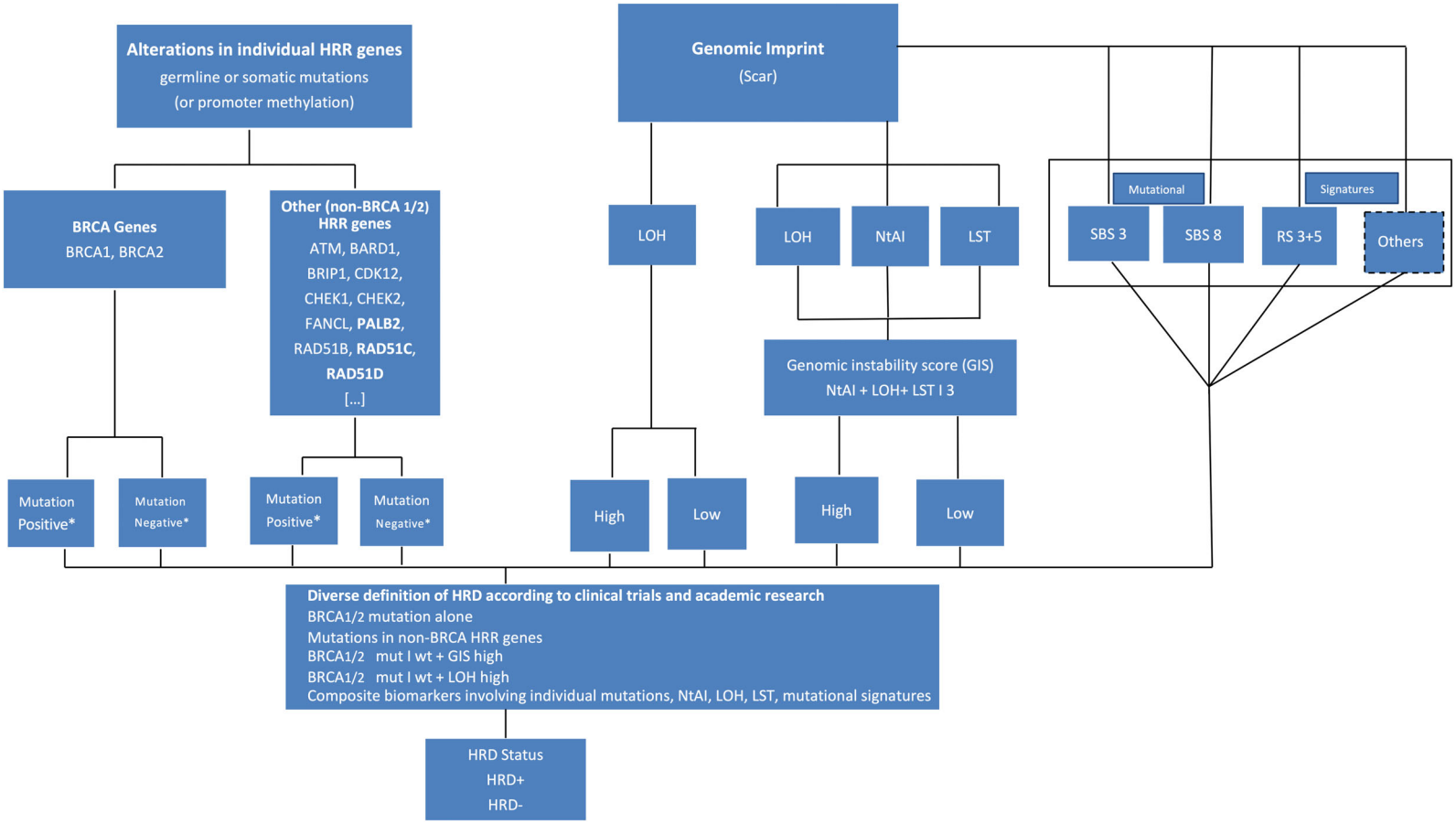


**FIGURE 1** Overview on parameters leading to the detection of homologous repair deficiency (HRD). One approach of analyzing HRD is the identification of loss of function mutations (pathogenic or likely pathogenic mutations according to ACMG criteria[15]) in *BRCA1/2*. These are the best characterized causes of HRD. The biological and clinical impact of loss of function mutations in other (non-*BRCA1/2*) HRR coding genes is less clear as is the role of promoter methylation. Further research is warranted in this field. Several clinical trials followed the approach of identifying HRD causing mutations in individual HRR genes. More recent developments in clinical trials and academic research investigate the consequences of these mutations: specific imprints in the genome ('genomic scar') are used to identify tumors with HRD. Of note this 'scar' is always historic and may not reflect the actual status of the tumor: reversion mutations may reinstate HR proficiency although the scar is still present. Current clinical trials use a composite biomarker approach where an integrated analysis of several different types of imprints in conjunction with the assessment of *BRCA1/2* mutational status indicates HRD. LOH, loss of heterozygosity; LST, large scale transition; NtAI, number of sub-chromosomal regions with allelic imbalance extending to the telomere, asterisk: according to ACMG criteria[15]

but can quickly become challenging when analyzing genes or specific mutations in genes for which only limited data are available.

While the detection and analysis of aberrations in individual or a set of HRR genes aim at identifying the cause of HRD, an alternative strategy is for example the identification of the consequences of a deleterious mutation in *BRCA2* in a tumor genome (Figure 1). This is because over the course of disease, tumors with loss of function mutations in one or more HRR genes can develop several types of recurrent and specific genetic imprints ("genomic scar"), which can be read out by genomic analysis. Of note, these imprints reflect the past (the history of the tumor), but not necessarily the current HRR status of the tumor, which may re-acquire HRR proficiency through a reversion mutation[16] in an HRR gene previously carrying a loss of function mutation. This pitfall needs to be kept in mind when looking at a clinical report that details such genomic scars. Typical read-outs of these scars involve loss-of-heterozygosity (LOH),[17] the number of subchromosomal regions with allelic imbalances extending to the telomere[18] (NtAI) and large scale transitions (LST) alone.[19] An alternative strategy is an integrated combinatorial analysis of three parameters (composite biomarker) resulting in a genomic instability score (GIS),[19] which are further combined with data on the mutational status of *BRCA1* and *BRCA2*. Both, the LOH-based composite biomarker and the GIS-based composite biomarkers are used in clinical trials and have been recently approved as diagnostic tests in conjunction with *BRCA1/2* analysis in routine care.[20-23] Other approaches rely on mutational signatures originally identified by Alexandrov et al.[24,25] As demonstrated by the group of Nik-Zainal[26] a whole genome sequencing (WGS)-based multiparametric model involving multiple layers of genomic information can significantly improve identification of HRD cases beyond currently used composite biomarkers. This clearly illustrates the power of academic-driven developments in the field of diagnostic testing and suggests that assays approved in clinical trials leave room for optimization. The colleagues around Daniel Hübschmann's group[27] developed the bioinformatics tool YAPSA that allows to infer a multitude of mutational signatures from comprehensive genomic data supporting not only research projects but also the implementation of more complex biomarker approaches in the field of HRD and beyond. Of note, assays designed to infer genomic HRD scars require a significant genomic footprint that well extends currently available large panels (which for example, are in use for TMB analysis).[28]

About a decade after the discovery of *BRCA1*[29,30] and *BRCA2*,[31] inhibitors of the enzyme Poly-ADP ribose PARP, which is implicated in several DNA repair pathways, were found to be effective in BRCA-deficient cells.[32,33] The reason for this is the mechanism of action of PARP inhibitors, which trap PARP1[34] on to DNA harboring single strand breaks interfering with the DNA replication machinery and subsequent generation of a double strand break which cannot be repaired by an HRR deficient cell. After these seminal discoveries, rapid drug development programs led to clinical trials that aimed at investigating synthetic lethal therapies in ovarian cancer,[35] quickly expanding the landscape of therapeutic options in several lines of therapy. Foo and colleagues[36] summarize the history and provide guidance in an increasing landscape of clinical trial data and testing scenarios. Other major cancer types where PARP inhibitors and combinatorial therapies are being developed and recent trials showed encouraging results include breast, pancreatic and prostate cancer. Reviews by Ali et al.,[37] Singh et al.[38] and Nientiedt et al.[39] thoroughly describe recent clinical and diagnostic data in these cancer types and provide an outlook on future developments. Future clinical trials will show whether this therapeutic approach can be extended to other cancer types, possibly also in conjunction with other therapies including checkpoint blockers.[40] This being said, further developments will only be successful if we agree on a uniform definition of HRD in a clinical context. The scientific community needs to obtain an even deeper scientific understanding of HRR biology including the specific impact of mutation types in individual non-*BRCA* HRR genes, the impact of somatic vs germline variants, the role of promoter methylation and implications of zygosity in this context. We might also consider a re-analysis of our current classification of variants in HRR genes in the context of therapy response prediction. A more thorough analysis of HRR in the context of different cancer types will help answering the question whether HRD has the same biological and clinical implications across cancer types and whether a one-test -one-score fits all approach is the optimal solution in a diagnostic setting. One avenue that may provide answers to some of these questions is the assembly and careful integrated analysis of multiple datasets, from clinical trials and the scientific community.

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## DATA AVAILABILITY STATEMENT

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## ORCID

*Albrecht Stenzinger* https://orcid.org/0000-0003-1001-103X

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