



Review Article

# A brief overview of homologous recombination deficiency testing in cancers for the ‘Next-Generation’ Pathologist

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## ABSTRACT

Genomic instability is one of the hallmarks of cancer, having a crucial role in cancer pathogenesis as well as tumor proliferation. This essential feature is secondary to dysregulation of DNA damage repair pathways. Homologous repair represents the most reliable double-strand break repair mechanism. Homologous recombination deficiency is responsible for generating and perpetuating DNA damage in cancer, posing an opportunity for targeting treatment with poly(ADP-ribose) polymerase inhibitors through ‘synthetic lethality’, as well as platinum-based agents. Comprehensive genomic analysis has made it possible to discover molecular biomarkers that assist in the identification of Homologous recombination deficient tumors, allowing for the expansion of such treatment strategies to various other malignancies. Leveraging the improvement of genomic analysis methods to be more efficient in identifying Homologous recombination deficiency is crucial in the advancement of cancer care. The current review highlights the current strategies for Homologous recombination deficiency detection, clinical implications, limitations, and applicability.

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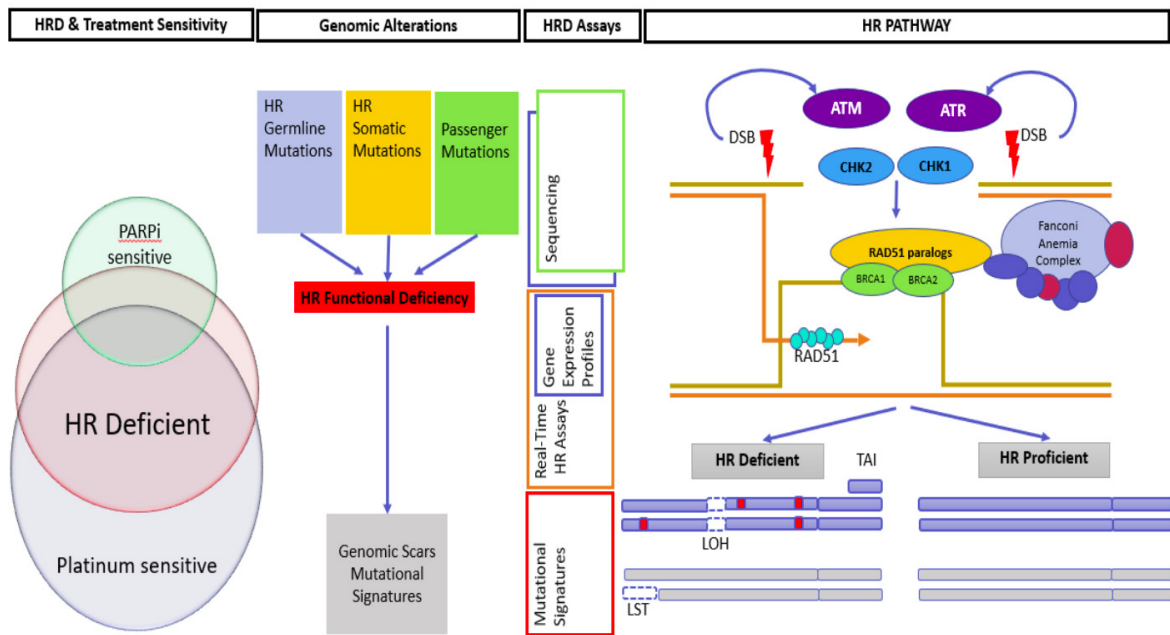
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## INTRODUCTION

Cancer development is associated with oncogenic transformation led by genomic instability.<sup>1</sup> Multiple genomic events are responsible for this transformation including mutations, copy number changes, and chromosomal changes, which can occur independently or conjunctively. Dysregulation of DNA damage repair (DDR) represents a fundamental feature of cancer pathogenesis and progression.<sup>1</sup> This is in concordance with the estimation that two-thirds of cancer mutations occur due to DNA replication errors.<sup>2</sup> DDR mechanisms include homologous recombination (HR) for the repair of double-strand breaks



**Figure 1.** Homologous repair (HR) pathway is activated after double-strand breaks (DSB), activating multiple proteins sequentially (ATM, ATR, CHK1/2, RAD51, BRCA1/2, Fanconi anemia complex genes) to allow DNA high fidelity repair. Genomic alteration in the HR pathway leads to HR functional deficiency (HRD) causing loss of heterozygosity (LOH), large-scale transition (LST), telomeric allelic imbalance (TAI), establishing genomic scarring and unique mutational signatures. These are identifiable through multiple HRD assays, sequencing, gene expression profiles, real-time HR assays. Although there is considerable overlap between HRD status, platinum chemotherapy, and PARPi sensitivity, HRD is not an exclusive parameter for the prediction of response to these agents.

(DSB) among various other mechanisms.<sup>3-5</sup> Consequently, homologous recombination deficiencies (HRD) result in distinctive genomic alterations arising as a result of inherited and/or somatic mutations, that determine both genomic scarring and unique mutational signature patterns in various cancers.<sup>3-5</sup> Recent advancements in genomic technology have helped capture these HRD related genomic scars and mutational-signatures to a certain extent and the determination of such ‘tumoral HRD status’ has been proven useful in driving treatment decisions.<sup>3-5</sup>

**TARGETED THERAPEUTIC APPLICATIONS OF HOMOLOGOUS RECOMBINATION DEFICIENCY IN CANCERS**

DNA repair pathways are indispensable to maintain DNA integrity and genomic stability in healthy tissues, and for resolving potentially lethal DNA damages.<sup>2</sup> DNA DSBs are preferentially repaired by high fidelity HR pathway, while single-strand breaks (SSB) are repaired by the base excision repair (BER) pathway.<sup>6</sup> PolyADP-ribose Polymerase1 (PARP1) protein is critical for SSB repair via the BER pathway.<sup>6</sup> BRCA1/2 genes encode for critical HR pathway proteins.<sup>6</sup> Consequently, BRCA1/2 mutated tumors are HR deficient and are unable to repair damaged DNA.<sup>6</sup> In this context, the event of PARP1 inhibition will lead to SSBs accumulation, eventually causing DSBs, which ultimately results in cell death due to the failure of the HR repair mechanism.<sup>6</sup> This strategy of using PARP inhibitors to

induce cell death in HR deficient tumors is called ‘synthetic lethality’.<sup>6,7</sup> Successful implementation of this therapeutic approach has been demonstrated in BRCA1/2 deficient breast, ovarian and prostatic cancers.<sup>7-9,11-15</sup>

Recent studies have demonstrated that in sporadic cancers, apart from BRCA1/2 mutations, dysregulation of any of the other critical proteins that make up the HR repair pathway such as RAD51, ATR, ATM, CHEK1, CHEK2, and Fanconi anemia complex genes (FANCD2, FANCA or FANCC 2) also causes HRD and confers such tumors with ‘HRD status’ or so-called ‘BRCAness’ phenotype (i.e. those tumors that may not have BRCA1/2 loss but show genomic alterations consistent with functional BRCA1/2 loss).<sup>6,8,11</sup> Tumors with this BRCAness phenotype have been documented to benefit greatly from treatment with PARPi.<sup>6,8</sup> Figure1 shows a summary of the HRD mutational landscape including the various genes involved in the HR pathway, existing methodologies to detect the genomic alterations resulting from interruptions to the HR pathway, and the dynamics between HRD and treatment sensitivity to chemotherapeutic agents such as PARPi and platinum agents.<sup>11</sup> Thus, it is clear that there exists a considerable overlap in the HRD targeting landscape and this can be leveraged to extend such treatment strategies to various other cancers that demonstrate HRD beyond familial breast/ovarian cancers.

## STRATEGIES TO DETECT HOMOLOGOUS RECOMBINATION DEFICIENCY

Defects in DNA repair processes can lead to specific patterns of genomic alterations that can indicate a defect in a specific repair pathway or can lead to abnormal gene expression patterns.<sup>10,11</sup> Many tumors harbor gross chromosomal rearrangements as a result of defective DNA repair and therefore assays have been developed to detect these so-called ‘genomic scars’, likely left behind by the loss of homologous recombination function.<sup>10,11</sup> Different approaches are currently being investigated to identify HRD in tumors such as quantifying the extent of these genomic scars or by analysis of mutational signatures in specific tumors.<sup>10,11</sup> Current strategies that are useful for detecting these genomic alterations employ high-throughput techniques for single nucleotide polymorphism (SNP) based genomic assays and molecular signature analysis.<sup>10,11</sup>

### SNP-BASED GENOMIC SCAR ASSAY

Three types of SNP-based genomic assays are currently available to assess genomic scars in cancers, they include loss of heterozygosity (LOH), telomeric allelic imbalances (TAI), and large-scale transitions (LST).<sup>11</sup> Each of these quantifies a distinct type of genomic scar that is related to defective DNA repair.<sup>11</sup> Thus, they can be considered as biomarkers for the state of DNA repair in particular cancer. Marquard et al analyzed the genomic scores of these biomarkers in 5371 tumor specimens across 15 cancer types and their study revealed a good correlation between the individual scores of the three biomarkers in different tumor types. Interestingly, cancers, where platinum therapy was the standard of care, showed significantly increased genomic scar scores. Additionally, they also found the presence of small subgroups of tumors with high genomic scar scores even in cancers where platinum was not the standard of care.<sup>18</sup> Thus suggesting the potential therapeutic application of platinum chemotherapy in certain subgroups of patients with cancers that were not conventionally treated with platinum.<sup>18,19</sup>

Myriad Genetics offers ‘myChoice HRD’ test to detect genomic instability status in primary/metastatic breast and ovarian tumor samples.<sup>19</sup> This test uses genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue.<sup>19</sup> Genomic instability status assay is a hybridization capture panel that targets SNPs distributed across the whole genome.<sup>19</sup> Next-generation sequencing (NGS) libraries are hybridized to this panel to enrich for sequences spanning these SNP locations and are sequenced using Illumina HiSeq next-generation sequencer.<sup>19</sup> From the sequencing reads allele-specific copy number profiles are generated which are used for calculation of genomic instability score.<sup>19</sup> This score takes into account LOH, TAI, and LST. The results are presented as an ‘HRD score’

and tumors with scores above a validated cut-off ( $\geq 42$ ) are termed ‘HRD positive’.<sup>19</sup> This is useful in predicting response to neoadjuvant platinum chemotherapy in triple-negative breast cancer (TNBC).<sup>20</sup> The NOVA study investigated the usefulness of the PARPi niraparib in platinum-sensitive ovarian cancer and found that patients with high HRD scores benefitted from it.<sup>21</sup>

### MUTATIONAL SIGNATURE ANALYSIS

Somatic mutations can result from a plethora of exogenous (environmental) or endogenous (impaired DNA damage respond) events and each of these leave behind its unique imprint on the cancer genome called ‘mutational signatures’.<sup>19</sup> Detecting and identifying mutational signatures have proven to be useful in guiding therapeutic decisions in various cancers.<sup>19</sup> The availability of genome-wide mutational data across cancer genomes is very important for mutational signature analysis and NGS technologies have emerged as an important tool in this context.<sup>19</sup> The capacity of NGS to analyze cancer genomes comprehensively has enabled the generation of comprehensive catalogs of somatic mutations in cancer patients.<sup>19</sup> The most studied method of analysis of mutational signatures are based on base-pair substitutions and are characterized by the specific base change and its direct 5’ and 3’ flanking base.<sup>11,19</sup> There can be 96 different combinations of trinucleotide changes given that there are six classes of base substitution and 16 possible sequence contexts.<sup>19</sup> The mutational signatures can be deciphered from large volumes of NGS data from cancer patients by computational algorithms that recognize recurrent patterns using the ‘96-mutation matrix’.<sup>19</sup> Thus, each pattern represents the relative proportion of each trinucleotide change, which translates to a mutational signature.<sup>19</sup> In addition to base substitutions, other mutational events like indels, copy number variations (CNV), and structural rearrangements also have to be taken into account while analyzing the pattern of mutational signatures.<sup>19</sup> Figure 2 provides a simplified overview of the utility of mutational signature analysis in the clinical setting. Figure adapted from Hoeck et al, 2019.<sup>19</sup>

Patient diagnosed with cancer undergoes biopsy from the tumor as well as from healthy tissue (e.g blood). Whole Genome Sequencing (WGS) technology is employed to sequence the DNA from both the samples for characterization of base substitutions, indels, structural rearrangements, and copy number variants (CNVs). The DNA from the healthy sample is used to characterize predisposition variants, while the cancer DNA sample provides for the characterization of medically actionable somatic driver mutational events. Thus a mutational signature pattern is identified, which either can directly support a specific cancer diagnosis in certain cases or influence clinical decisions such as referral for genetic counseling services, screening for family members, patient-specific treatment protocol selection, or prognostication.

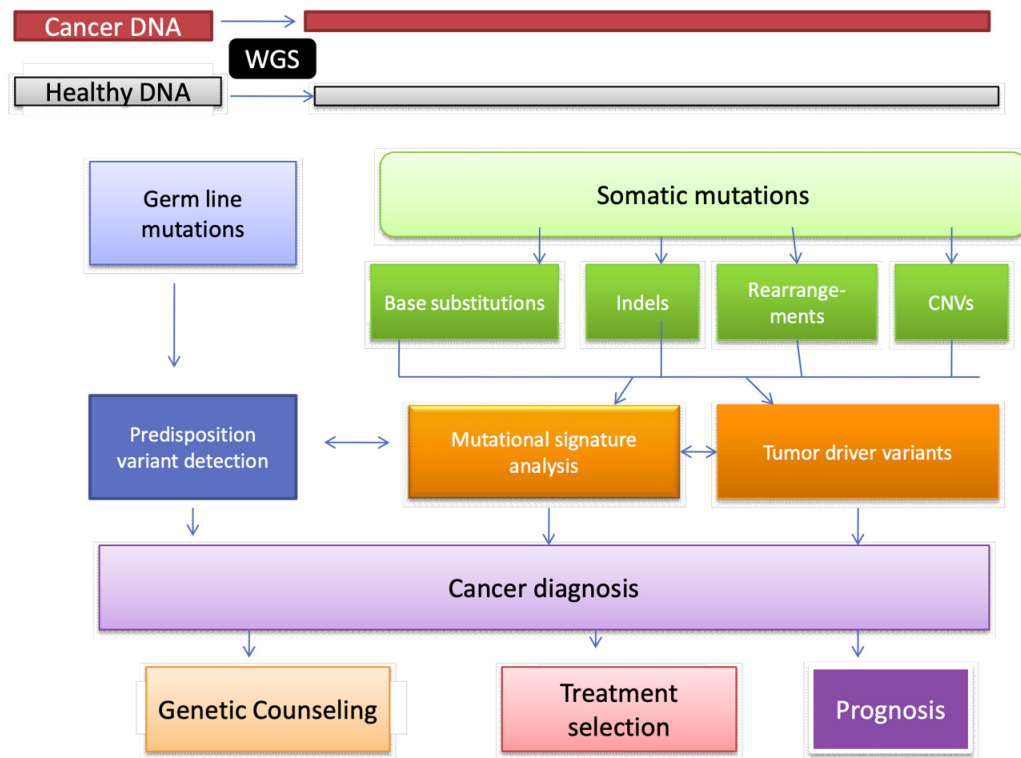


Figure 2<sup>19</sup>: Overview of mutational signature analysis as a cancer diagnostic tool:

‘Signature 3’, first described by Alexander et al, is one such mutational signature that is attributable to HRD and is characterized by relatively equal proportions of all possible trinucleotide changes.<sup>3</sup> It has been documented to exist in cancers involving many organs including breast, ovary, pancreas, and prostate.<sup>3</sup> Currently, mutational-signature analysis based diagnosis is popular in the context of breast and ovarian cancers because of the well-documented evidence that BRCA1/2 loss in tumors correlates with HRD and thus renders them amenable to treatment with PARPi or DSB-inducing agents (platinum).<sup>19</sup>

Computational algorithms such as ‘HRDetect’ have been developed to accurately detect HRD in breast cancers based on their mutational signatures.<sup>21</sup> HRDetect requires data from whole-genome sequencing. It needs inputs of the following information: mutational signature counts for each single base substitution, information on indels, and HRD score (sum of LOH, TAI, and LST scores).<sup>21</sup> Using a machine learning algorithm (lasso regression), it assigns weighted values to these parameters and aggregates them into a single final score which predicts the degree of BRCA1/2 deficiency.<sup>21</sup> In addition to identifying BRCA1/2 deficient tumors, HRDetect can identify tumors with ‘BRCAness’ with a sensitivity of almost 100%.<sup>19</sup> Thus, mutational-signature analysis based tests such as HRDetect have the potential to find application in selecting patients who may benefit from treatment with PARPi or platinum agents in not just BRCA1/2 mutated breast/ovarian cancers but also various other cancer types associated with ‘BRCAness’.

## ROLE OF CIRCULATING TUMOR DNA IN HOMOLOGUS RECOMBINATION DEFICIENCY DETECTION

Targeted sequencing of circulating tumor DNA (ctDNA) is a minimally-invasive technique, wherein tumor cells circulating in the peripheral blood are isolated and analyzed for genomic aberrations.<sup>22</sup> This strategy is now being increasingly used for early detection and monitoring progression in various cancers. Barbacioru et al, recently, developed a unique statistical method to identify the presence of LOH or somatic biallelic copy number loss of BRCA1/2 in ctDNA.<sup>23</sup> The model was developed using coverage profiles and allelic frequencies of SNPs for each gene of interest and validated using in-silico tools.<sup>23</sup> The model was applied to ctDNA samples of a large cohort of patients with advanced solid tumors and the results of this study were found to be highly concordant with the results from matched tissue samples.<sup>23</sup> Another group demonstrated the utility of ctDNA in detecting clinically actionable genomic alterations in ovarian cancers.<sup>22</sup> A few clinical trials have used targeted sequencing of ctDNA for detecting HRD status in advanced prostatic cancers.<sup>6</sup> Thus ctDNA-based strategies have great potential for clinical applicability in longitudinal monitoring of patients with advanced cancers given their minimally-invasive nature.

## CONCLUSIONS

It is clear that HRD plays a crucial role in cancer

pathogenesis and progression.<sup>4</sup> Hence, accurate estimation of HRD status is essential, not only to guide treatment decisions but also for the development of novel therapeutic strategies, with the ultimate objective of expanding the pool of patients who may derive clinical benefit from such approaches. Although current approaches such as genomic scar assays and mutational signature analysis represent useful tools for determining tumoral HRD status, many of them are in their early stages of development requiring further validation studies. Therefore, there is an urgent need to further develop reliable HRD detection methodologies that are comprehensive, cost-effective, and minimally-invasive with a high predictive value for treatment response and disease progression.

**Conflict of interest: None**

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