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**Homologous Recombination Deficiency in Breast Cancer: implications for risk, cancer
development and therapy**

Abbreviated title: HRD in breast cancer

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Abstract

An underlying cause of breast cancers has been largely attributed to defects in the DNA damage response (DDR) pathway. In particular, the homologous recombination (HR) pathway repairs double-stranded breaks (DSBs) in DNA, ultimately protecting the cell from genomic instability and thus preventing the accumulation of transforming mutations. In line with this, mutations in a number of genes encoding HR proteins are a well-studied cause of HR deficiency (HRD), and, at the germline level, can confer risk to breast cancer but also

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occur somatically, contributing to sporadic breast cancer development, progression and response to therapy. Our understanding of the biological processes involved in HR and how these become compromised during breast cancer development has led to a better understanding of how HRD cells can be targeted with specific DNA damaging agents and/or with synthetic lethal targeting approaches such as PARP inhibition. Additionally, *in vitro* and preclinical modelling has supported the development of clinical trials to assess targeted therapies such as PARP inhibitors (PARPis), ultimately leading to development of therapies with greater clinical benefit. A number of challenges have been encountered, including resistance to therapy; however, addressing these challenges head-on and continually driving scientific research and clinical trials with innovative therapies will contribute to our ability to target HRD in breast cancers. Ongoing research efforts into HRD in breast cancer development are therefore essential, even in the era of targeted therapies, to provide innovative strategies for improved tumour responses.

Introduction

The cellular genome is continually exposed to DNA damaging events, including endogenous sources such as oxidation, alkylation and DNA base mismatches, as well as exogenous sources including ultraviolet (UV) radiation, ionizing radiation (IR) and other chemical agents including chemotherapeutics. Indeed, organisms have developed an armoury of defence pathways to overcome these DNA insults. This includes sensor proteins that detect DNA damage, transducers that mediate and/or amplify this information/signal and effectors, which regulate numerous cellular processes such as DNA damage checkpoint control, transcriptional reprogramming, RNA processing, DNA repair and apoptosis. Ultimately, this orchestrated process plays an essential role in detecting DNA lesions, signalling their presence and promoting their repair via DNA damage response (DDR) pathways, facilitating DNA repair and the maintenance of genomic integrity.

The major DNA repair pathways within human cells include direct damage reversal, base excision repair (BER), nucleotide excision repair (NER), DNA mismatch repair (MMR), inter-strand crosslink repair (Fanconi anaemia (FA)/BRCA), and the double strand break repair pathways homologous recombination (HR) and non-homologous end joining (NHEJ)¹. DNA double-stranded breaks (DSBs) are considered the most severe DNA lesions, as these can result in loss and/or amplification of genetic information which, with the exception of some well characterised gain-of-function point mutations, are the main drivers of cancer, particularly breast cancer. HR encompasses a number of interrelated pathways that utilise the homologous sister chromatid for template-directed error-free DNA repair of DSBs, whilst NHEJ involves repair of DSBs via re-ligation of the DNA ends (potentially resulting in repair errors)².

A defect in any of these pathways can lead to genomic instability and mutagenesis, whereby the cell is unable to repair damaged DNA effectively. As genomic instability is a hallmark of

cancer^{3,4}, it is unsurprising that the development of cancer is a consequence of DDR pathway defects, including HRD. Indeed, it has been estimated that up to 40% of familial and sporadic breast cancers have an HRD⁵, and understanding the link between HRD and breast cancer development at the molecular level has been crucial in developing targeted therapies for breast cancer treatment and understanding tumour response. Here, the role of HRD in breast cancer risk, development and treatment response is reviewed.

Homologous Recombination DNA Repair Pathway

As highlighted above, HR is a relatively error-free DNA repair pathway, utilising a sister chromatid as a template for copying and replacing damaged DNA. Consequently, repair of DSBs via HR can only occur in the S and G2 phases of the cell cycle, during which a homologous sister chromatid is available.

Specifically, at the outset of HR, DSBs are sensed by a trimeric complex known as the MRN complex. This complex is composed of meiotic recombination 11 (MRE11), the DNA repair protein RAD50 and Nijmegen breakage syndrome 1 (NBS1)⁶. This initial sensing of DSBs is performed in combination with the human single strand binding protein (hSSB1) (the ‘sensor’ proteins) which directly binds to ends of DSBs tethering the MRN complex to broken DNA ends. MRN then recruits and activates the checkpoint kinase ATM (Ataxia Telangiectasia Mutated), which in turn phosphorylates a number of substrates involved in the signalling of DNA damage (‘mediators’), at specific residues⁷. For example, at, and surrounding, the DSB site, ATM phosphorylates the histone protein H2AX within the chromatin at serine 139 (Ser139) forming γ H2AX. The mediator of damage checkpoints protein 1 (MDC1) binds directly to γ H2AX through its phosphoprotein interacting BRCT domain and recruits and helps activate additional ATM at the break site, thus amplifying and propagating the DDR signal². Additionally, Chk1 and Chk2 (‘transducers’), which are directly phosphorylated and activated by ATM and the ATM and Rad3 related kinase ATR, are also involved in the signalling of DSBs and directly initiate cell cycle arrest to allow time for repair^{8–11}. Structural changes in the chromatin surrounding DSBs also result in the binding of the pro-NHEJ factor 53BP1 to methylated histones surrounding the DSB site. Finally, the breast and ovarian cancer susceptibility protein BRCA1 associates with C-terminal binding protein interacting protein (CtIP), which then further interacts with the MRN complex¹². Once the necessary proteins have been recruited to the DSB site, DNA end resection begins. This is performed

by the MRN complex, and more specifically, by the Mre11 nuclease. Mre11 dependent DNA end resection requires CtIP and is catalysed by BRCA1, which promotes the removal/remodelling of 53BP1, a resection blocking protein, away from the DSB ends, thus facilitating HR mediated DSB repair. The combined efforts of MRN, CtIP and BRCA1 result in resection of the 5' DNA strand, resulting in a protracted 3' single stranded DNA (ssDNA) overhang. This 3' ssDNA, is rapidly bound/coated with replication protein A (RPA) to form a nucleoprotein filament, thus protecting it from degradation. Then, RPA is displaced through the recruitment of partner and localiser of BRCA2 (PALB2) which in turn recruits BRCA2, which then loads the recombinase RAD51 to replace RPA in a reaction promoted by mediator proteins, such as XRCC2, XRCC3 and RAD52 amongst others. This generates the Rad51-ssDNA-nucleoprotein filament that invades the homologous site within the double-stranded sister chromatid¹³. At this site, a transient displacement loop (D-loop) is formed which functions as a transient replication fork, allowing the replication machinery to replace damaged/resected DNA in an error free manner, after which the resultant Holliday junction is resolved, and the error-free repair of DNA is complete. This process is summarised briefly in

Figure 1.

HR Defects and Breast Cancer

It is evident that a number of proteins play a key role in the repair of DSBs by HR. Indeed, the importance of the breast and ovarian cancer susceptibility proteins BRCA1 and BRCA2 has been well documented, with the first suggestion of a role for BRCA1 in HR, due to its co-localisation with Rad51 following DNA damage, reported in 1997 by Scully et al^{14,15}. In line with this, Moynahan and colleagues demonstrated that BRCA1^{-/-} mouse embryonic stem cells had a 5- to 6-fold lower level of HR activity than BRCA1^{+/-} cells¹⁶. A role for BRCA2 in HR was reported soon after, as BRCA2 contains a DNA binding domain (DBD) capable of interacting with dsDNA as well as RAD51, with early phenotypic studies showing BRCA2 depletion can lead to heightened sensitivity to DNA damaging agents and impaired HR following DNA damage¹⁷⁻²¹. Indeed, it is essential that the genes encoding these proteins function appropriately. For example, any mutations within these genes or genomic regulatory regions e.g. promoters/enhancers, may prevent optimal expression of these genes or the function of their encoded protein, thus affecting synthesis of functional HR proteins, leading to HRD and abnormal DSB repair. As a result of this deficient/abnormal DSB repair, HRD results in the accumulation of mutations/aberrations, ultimately leading to heritable genomic “scars” within HRD cells/tumours.

A number of factors can result in HRD (**Figure 2**). For example, epigenetic modification of the HR genes, such as inactivation of *BRCA1* or *RAD51C* by promoter hypermethylation leading to downregulation of gene mRNA expression, has been shown to correlate with HRD^{22,23}. A number of structural rearrangements may also lead to HRD. Telomeric allelic imbalance (TAI) is the unequal contribution of maternal and paternal DNA, whereby breakpoints in TAI regions can lead to imbalances and HRD²⁴. This is part of the characteristic ‘genomic scar’ caused by HRD, which also includes loss of heterozygosity (LOH)²⁵ and large-scale state transitions (LST)²⁶. LOH is a frequent genetic event in many

cancer types, during which a change in polymorphic markers from a heterozygous state in the germline to a homozygous state in the tumour DNA occurs²⁷. LOH can involve copy number loss, whereby all or part of a chromosome has been deleted and has been identified on a number of chromosomal arms in breast cancer²⁸.

However, the most commonly described cause of HRD in the literature are deleterious mutations within the coding regions of genes encoding the core HR proteins (**Figure 2**) and such mutations may be either germline or somatic. Germline mutations in *BRCA1* and *BRCA2* are the most well characterised cause of HRD, and the most common cause of hereditary breast and ovarian cancer (HBOC)²⁹. Although *BRCA1/2* variants confer a high risk of developing breast cancer (with a relative risk of 5 or higher), it has been estimated that only 4.6% of women have mutations in these genes, demonstrating that mutations in these genes are relatively rare in the general population^{30–32}. Nonetheless, because the risk of breast cancer conferred by a germline *BRCA1/2* mutation is so high, *BRCA1/2* are classified as high-penetrance breast cancer genes (**Table 1**)^{33–35}. Such *BRCA* mutations can downregulate HR, consequently favouring more error-prone DSB repair pathways (such as NHEJ). This may result in the introduction of mutations or large deletions in the DNA (the latter of which may include LOH), ultimately increasing the acquisition of tumorigenic structural rearrangements and genomic instability, leading to breast cancer (**Figure 2**).

It is important to note that the *BRCA* genes do not explain the full extent of clinically relevant HRD in breast cancer. Indeed, breast tumours lacking *BRCA* mutations but with HRD have been described, and implicated genes include those encoding proteins involved in DSB repair, which interact with *BRCA1/2* during the HR process. HR genes with an established role in significantly increasing breast cancer risk include *PALB2*, *ATM* and *CHEK2* (**Table 1**), which are part of a group of genes that increase breast cancer risk by approximately 2- to 5-fold³², and are consequently classified as moderate-penetrance (or moderate-risk) genes.

However, Antoniou et al suggested that variants in *PALB2* confer a five-fold or higher risk of breast cancer⁴², and both Slavin et al and Thompson et al classified *PALB2* as a high risk gene for hereditary breast cancer^{32,50}. Subsequently, it is now accepted that *PALB2* is a high, rather than moderate risk, breast cancer gene.

Additional genes in the HR pathway, such as *MRE11*, *RAD50* and *NBS1* of the MRN complex and *RAD51* have been investigated for breast cancer risk,⁵¹ with a number of genes encoding the RAD51 paralogues, such as *RAD51B* and *RAD51C* being implicated. However, the level of risk associated with mutations in these genes is not clear, with conflicting findings reported. De Leeneer et al and Le Calvez-Kelm et al found that deleterious variants in *RAD51C* do not increase breast cancer risk^{52,53}. Yet, a study by Jønson et al describes pathogenic *RAD51C* variants and several variants of the gene, arguing that these should merit consideration of *RAD51C* as contributing to breast cancer risk⁵⁴. This and other studies investigating the variants in *RAD51* and their association with breast cancer risk^{32,55–58} illustrate the need to continue investigations in some of the HR moderate risk genes to further understand their contributions to breast cancer risk.

Mutations in these HR genes often result in decreased gene expression and consequently alter protein levels and function. This may lead to decreased HR and a shift towards more error-prone methods of DSB repair, or to increased expression of HR genes. This latter situation may also prove advantageous to the balance of the HR pathway. For example, past studies have overexpressed RAD51 paralogues (yeast and human, including RAD51D) in the context of mutants deficient in these paralogs. These studies illustrated that overexpressing RAD51D was able to rescue the HRD in the mutants such that they were resistant to DNA damage^{59–62}. On the other hand, elevated RAD51 levels have been observed in breast cancer cell lines and primary tumours^{63,64} suggesting that driving the HR pathway above physiological levels may also play a role in genomic instability and hence tumour progression⁶⁵.

Additionally, certain HR genes have been associated with development of particular breast cancer subtypes. Clinically relevant breast cancer subtypes are detailed in **Table 2**. It is well-known that triple-negativity correlates with *BRCA1* status⁷⁰. In a recent case-control study by Hu et al, *BRCA1*, *BRCA2* and *PALB2* mutations were particularly associated with ER- and HER2- subtypes of breast cancer⁷¹ – also described in **Table 2**. *TP53* (which encodes a tumour suppressor, controls cell cycle and activates DNA repair proteins such as RAD51) mutations, were shown to be enriched in the HER2+ subtypes – a finding also corroborated by Slavin et al in 2017^{32,36,37,72}. *ATM* and *CHEK2* mutations were associated with all subtypes except for triple negative breast cancers (TNBCs) with *RAD51C* and *RAD51D* mutations enriched in ER- tumours only. In 2018, Shimelis et al performed a 38 gene hereditary cancer panel test in 10,901 TNBC patients and revealed that germline pathogenic variants in the HR genes, including *BRCA1* and *RAD51D*, were associated with a high risk of TNBC. Pathogenic variants in *BRIP1*, *RAD51C* and *TP53* were associated with moderate risk of TNBC⁷³. Studies like these can help clinicians guide subtype-specific breast cancer risk and improve patient management e.g. through the use of anti-oestrogen medication for chemoprevention in women at high risk of developing ER positive disease.

HR Defects and Cancer Treatment

For decades, anthracyclines (broad-spectrum anticancer drugs, which act by the inhibition of topoisomerase II) such as doxorubicin and epirubicin have been amongst the most widely used chemotherapeutic agents for breast cancer treatment. Anthracycline-containing chemotherapy regimens (so-called second generation regimens) have demonstrated survival benefits over combinations such as cyclophosphamide, methotrexate, and fluorouracil (CMF)^{74–78}. Furthermore, the use of taxanes (which act by binding to and stabilising microtubules to induce mitotic cell death), such as docetaxol and paclitaxel, in third generation treatment regimens, have been shown to be superior to anthracycline-containing second generation regimens alone in terms of overall survival^{79–81}. However, there are currently no good biomarkers available to predict response to particular chemotherapeutic agents and thus generally anthracycline-taxane regimens are used irrespective of molecular subtype.

A key question for clinicians is whether an understanding of the genetic aberrations in hereditary breast cancer can be exploited to improve patient outcomes. The genomic instability induced by mutations in HR genes potentially renders cancer cells susceptible to chemotherapeutic drugs, particularly those which act directly to damage DNA, such as anthracyclines or platinum^{82–88}. In support of this, a number pre-clinical studies have shown that BRCA1/2 deficient breast cancer cell lines, which lack functional HR, are much more sensitive to platinum-based drugs which induce inter-strand crosslinks^{89–98}. For example, Bhattacharyya et al showed that BRCA1^{-/-} mouse embryonic stem cells were 5-fold more sensitive to cisplatin when compared with wild-type cells⁹⁰. Confirming this, Fedier et al demonstrated that human breast cancer cells deficient in BRCA1 had an increased sensitivity to the platinum carboplatin and oxaliplatin⁹³. Cells from mice harbouring a deletion in exon 27 of *BRCA2* (the region to which RAD51 binds) were hypersensitive to mitomycin C (that

induces DNA inter-strand crosslinks), an expected observation considering the role BRCA2 plays in HR⁹⁵. Indeed, as this exon in *BRCA2* has been shown to maintain chromosomal integrity at stalled and collapsed replication forks during HR⁹⁶, cells lacking this exon would be expected to have an increased sensitivity to platinum-based drugs. Indeed, Yuan et al show that ectopic expression of a truncated BRCA2 protein (containing part of the Rad51 binding domain) increases sensitivity to cisplatin in vivo, presumably via a dominant negative interaction⁹⁸.

The clinical relevance of using platinum-based regimens in patients with a germline HRD has been explored in several clinical studies. Byrski et al in 2009 reported a small series of patients with pathogenic *BRCA1* mutations who received neoadjuvant treatment for breast cancer with cisplatin, reporting a pathological complete response (pCR) in 90% of patients⁸⁴. A further retrospective study from this research group reported similarly high pCR rates in *BRCA1* mutation carriers receiving neoadjuvant cisplatin (83%)⁸⁶. This finding was confirmed in a further study by Byrski et al in 2014, during which a pCR was observed in 61% of *BRCA1* mutant patients receiving neoadjuvant cisplatin⁹⁹.

Several prospective clinical trials have been conducted to evaluate the role of platinum in the treatment of breast cancer. These studies have often included unselected TNBC patients, although many have also stratified patients by germline *BRCA* mutation status. These studies are summarised in **Table 3**.

The TNBC Trial (TNT) phase III clinical trial compared carboplatin and docetaxol in unselected advanced TNBC. Overall, there was no difference in response rates (ORR) or progression-free survival (PFS) between the two groups. However, patients with a germline *BRCA1/2* mutation treated with carboplatin had both improved PFS (6.8 vs 4.4 months, $p=0.002$) and ORR (68% vs 33%, $p=0.03$) when compared to those treated with docetaxol¹⁰³.

In this study, the authors used an HRD assay (also referred to as the Myriad HRD assay, developed by researchers of Myriad Genetics Inc., that tests for the presence of structural rearrangements across the genome and outputs an HRD score to tumours¹⁰⁵) to identify patients with TNBC who potentially had genomic scarring arising as a consequence of an HRD, although patients with a high Myriad HRD score using this assay (unlike germline *BRCA1/2* mutation carriers) did not have a preferentially improved response to platinum¹⁰³.

In contrast, several studies have investigated the benefit of platinum to chemotherapy regimens in early breast cancer with a putative HRD. The PrECOG 0105/Cisplatin-1 and Cisplatin-2, and GeparSixto trials suggested a benefit for the addition of platinum in the neoadjuvant setting, in patients with HRD+ tumours, whether classified by the presence of a germline *BRCA1/2* mutation, or by the use of the Myriad HRD assay^{100,101}. However, the TBCRC030 phase II study was a phase II randomised comparison of neoadjuvant cisplatin or paclitaxel in patients with TNBC of either BRCA wild type or unknown mutational status, using a cut-off of Myriad HRD score ≥ 33 . HRD was not found to be predictive of pathological response to chemotherapy, and there was no difference in response rates between platinum or taxane¹⁰².

SWOG S9313 identified improved outcomes in patients with an HRD (either germline *BRCA* mutant or Myriad HRD ≥ 42), who were treated with adjuvant anthracycline/cyclophosphamide, when compared to patients without an HRD¹⁰⁴.

Overall, and notwithstanding some of the conflicting results from these clinical studies, there is a growing body of evidence that tumours with an HRD do derive a benefit from chemotherapeutic agents such as anthracyclines or platinum. The effectiveness of these agents is reflected in the role of *BRCA1/2* in DNA repair pathways, whereby germline mutations in either gene impair HR repair of DSBs and cause an HRD leading to genomic

instability. HR also contributes to repair DNA lesions (such as intra-strand crosslinks) induced by platinum-based cross-linking agents. Therefore, in the absence of functional BRCA proteins, cells are unable to repair these DNA breaks leading to apoptotic cell death of HR-defective cells (with a more limited effect on HR-proficient cells).

Whilst platinum agents may be relatively effective in treating *BRCA*-associated breast cancer, there may be significant associated toxicities. A recent meta-analysis of TNBC suggested that side-effects such as anaemia, thrombocytopaenia and nausea occurred more frequently in patients treated with platinum agents, who were also more likely to need dose reduction/or dose stopping due to toxicities¹⁰⁶.

Thus, targeted synthetic lethal approaches, such as those utilising poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) in BRCA deficient cells have shown great promise in the clinic for patients with a *BRCA1/2* breast cancer^{107–109}. PARP is an enzyme with a role in catalysing transfer of ADP-ribose to target proteins (including itself and many histone proteins), at SSBs within DNA. When PARP is inhibited, SSBs are not repaired, resulting in stalled replication forks during S-phase. The HR proteins, including BRCA1/2, are required to rescue these stalled forks. In the context of PARP inhibition and in the absence of BRCA1/2, DNA DSBs begin to accumulate during the S-phase (due to collapse of stalled replication forks). Consequently, this leads to apoptotic cell death of the HR-defective cells (with no effect on the HR-proficient cells). This concept is known as synthetic lethality (**Figure 3**). Indeed, PARPis are well tolerated because normal cells with at least one functional allele of *BRCA1/2* can overcome the effects of PARP inhibition, as they are able to rescue PARPi induced replication stress.

It was therefore hypothesised that PARPis will show clinical benefit in patients carrying a germline *BRCA* mutation, as such mutations may potentially predict response to PARPis

allowing clinicians to offer targeted therapy to germline *BRCA1/2* mutation carriers with breast cancer¹¹⁰. Consequently, PARPis have been the subject of investigation in several clinical trials assessing their benefit in *BRCA*-mutated breast cancer. Several studies in advanced breast cancer, including ICEBERG1, OlympiAD, EMBRACA and BROCADE3 have demonstrated benefit for PARP inhibition in advanced *BRCA*-mutated TNBC^{111–114}, and these studies are summarised in **Table 4**. On the basis of these results, the FDA approved the use of olaparib to treat patients with HER2 negative breast cancer in the presence of a germline *BRCA* mutation. The ongoing LUCY registry study is an open label single arm multicentre study evaluating the effectiveness of olaparib in improving PFS in patients with metastatic breast cancer and either a germline or somatic *BRCA* mutation¹¹⁵.

The benefit of adjuvant PARPis in *BRCA*-mutated early breast cancer, however, remains to be proven and is under investigation in the ongoing OlympiA trial, the results of which are awaited¹¹⁶.

A number of trials have investigated the potential benefit of PARPis in patients with unselected (*BRCA* wild-type or unknown mutation status) TNBC, hypothesising that a proportion of these tumours may harbour an HRD and thus the synthetic lethality resulting from PARP inhibition may be clinically beneficial. The NOBROLA, RUBY and TBB trials are phase II studies in advanced breast cancer evaluating olaparib, rucaparib and talazoparib respectively^{117–119}, with the first two studies showing some evidence of efficacy and the third ongoing. In early disease, the I-SPY2 study assessed the combination of neoadjuvant veliparib and carboplatin, reporting a pCR rate for this combination of 51%, compared with 26% in controls¹²¹. In contrast, the BrighTNess trial (comparing paclitaxel alone, paclitaxel plus carboplatin or paclitaxel, carboplatin and veliparib in unselected stage II-III TNBC) showed that while there was an increased pCR rate with the addition of carboplatin to

paclitaxel (31% vs 58%), no additional benefit was gained from adding PARP inhibition (pCR rate 53% in the veliparib arm)¹²⁰.

As a proportion of patients did not respond to PARPis in the OlympiAD and EMBRACA trials and the observed toxicities, it may be beneficial to refine groups of patients based on HRD status – who may be expected to derive an increased benefit from such therapies. Indeed, in the past 3 years, trials have been set-up to investigate this by incorporating HRD status into the inclusion criteria when assessing PARPi efficacy. These trials also tested these in non-*BRCA* breast cancers and are important as they can reveal roles of the other HR genes (carriers with HRD but no *BRCA* mutations are termed as ‘BRCAness’). Indeed, it has been shown that deficiencies in RAD51, ATM and CHK2 can induce sensitivity to PARPis¹⁰⁹.

Clearly then there is a requirement to identify breast cancers with an HRD, in order to exploit this therapeutically, and several putative biomarkers have been developed with this aim.

Indeed, there is significant data suggesting that the Myriad HRD assay discussed above may be predictive of patient benefit in the clinical setting. In addition, the HRDetect assay developed by Davies et al in 2014 was suggested to reveal a larger proportion of individual breast cancer patients with *BRCA1/2* deficiency than previously appreciated, who may benefit from PARP inhibition¹²².

The concept of ‘BRCAness’ has also been used to describe tumours with a potential HRD in the absence of a germline *BRCA1/2* mutation. In such tumours, mutations in other HR genes, methylation of the *BRCA1/2* (and perhaps other HR gene) promoters, or reduced expression of these genes via transcriptional regulation may explain an HRD when no mutations are identified¹²³. Indeed, non-*BRCA* HR gene mutated TNBCs were shown to share some of the ‘BRCAness’ features, such as a higher number and length of structural aberrations and may also benefit from PARP-inhibition¹²⁴.

A further potential biomarker for HRD has been described in the DNA Damage Response Deficiency) DDRD signature. This 44-gene, expression-based assay was developed using bone marrow derived from Fanconi Anaemia (FA) patients and from a cohort of *BRCA* mutant enriched breast cancers. This signature identified a subgroup of patients with a DDR deficiency (DDRD), characterised by upregulation of immune processes and as a result the assay has since been renamed the DNA Damage Immune Response (DDIR) assay¹²⁵. This signature has been validated as predictive of response to DNA-damaging chemotherapeutic agents in both the adjuvant and neoadjuvant settings^{104,125} and may have an advantage over other assays as it is reflective of the current biological response to HR deficiency within a tumour. In contrast, the HR assays discussed above, which detect genomic scarring caused by HRD, detect the non-reversible and heritable end-product of a HRD, which may be more reflective of tumour evolutionary history rather than current tumour biology. Indeed, a number of reports have demonstrated that *BRCA1/2* deficient HRD tumours can acquire further “reversion” mutations in *BRCA1/2* to become platinum and/or PARPi resistant, HR proficient, tumours. Whilst HR proficient, these tumours still harbour the genomic scars of their evolutionary HRD (the role of reversion mutations is discussed in further detail below). Interestingly, further investigation of the underpinning mechanism of the DDIR suggests that this may be directly linked to HRD, through accumulation of unrepaired/damaged DNA in the cytoplasm of HRD cells, resulting in activation of the cGAS/STING pathway. Sensing of cytosolic dsDNA by cGAS (Cyclic GMP-AMP (cGAMP) synthase) results in cGAMP production triggering, STING (stimulator of interferon genes) activation, causing activation of an IRF3/TBK1 dependent Type I interferon transcriptional cascade^{126–128}. This includes the upregulation of both chemokines and immune checkpointing genes, creating an “inflamed yet immunosuppressed” tumour microenvironment. This is borne out by the fact that the DDIR assay score has been shown to correlate with tumour infiltrating lymphocytes (TILs), yet in

the SWOG validation study by Sharma et al, DDIR outperformed TIL scoring in predicting response to adjuvant anthracycline chemotherapy¹⁰⁴.

Consequently, this assay provides a biomarker to identify breast cancers with an HRD where there is upregulation of immune checkpointing genes, which may then respond to treatment with immune checkpoint blockade, possibly in combination with DNA damaging agents. In line with this, Pantelidou et al have shown PARPi efficacy in *BRCA* mutant mouse models is dependent upon the STING pathway immune response¹²⁹. Additionally, early phase clinical work in the MEDIOLA basket trial has demonstrated anti-tumour activity for the combination of olaparib and durvulumab (a PD-L1 inhibitor) in germline *BRCA*-mutated advanced breast cancer¹³⁰. Furthermore, in the I-SPY 2 trial the use of this combination, together with paclitaxel, followed by doxorubicin/cyclophosphamide in the neoadjuvant setting in both high risk ER- and ER+ early breast cancer resulted in an estimated pCR rate of 37%, compared with 20% in patients receiving paclitaxel-adriamycin/cyclophosphamide chemotherapy alone¹³¹.

In conclusion, it is clear that a proportion of breast cancers harbour an HRD and this offers the opportunity to personalise treatment approaches, whether by selection of appropriate systemic cytotoxic chemotherapy, or the use of other targeted agents such as PARPis or immune checkpoint blockade. However, there remain challenges in identifying optimal treatment options and there clearly remains a need to better understand the interaction between HRD and therapeutics, and for improved biomarkers to advance treatment selection in these patients.

Challenges and future perspectives

It is clear from the above that HRD contributes significantly to breast cancer risk, tumour development and progression, and is also vulnerable to exploitation for therapeutic benefit in the clinical setting. Several studies have shown clinical activity of agents targeting HRD, such as platinum and PARPis, in both early and metastatic breast cancer, as discussed earlier. It is clear, however, that there remain significant challenges in the precision treatment of such cancers. Self-evidently, for agents targeting HR pathways, there needs to be clear evidence of HRD for these drugs to be effective. However, as the clinical data shows, there is no robust, reliable biomarker which accurately identifies tumours with an HRD, to allow treatment selection in the clinic. Although the Myriad HRD assay, and HRDetect have shown promise in this regard, the conflicting data reported above suggests that there is a need for better and more reliable biomarkers. In particular, these assays identify genomic scarring and therefore may be more reflective of the previous history of a cancer and its treatment, rather than its current biology. Furthermore, studies in advanced disease have used primary tumour tissue for these assays and it is known that primary tumour phenotype is not necessarily reflective of the biology of metastatic disease¹³². Thus, it is perhaps unsurprising that the use of such assays in this manner is not predictive of outcome in advanced disease. It has been demonstrated that the genomic landscape of metastatic breast cancer is significantly more disordered than early disease and that such changes may occur under treatment selection pressures¹³³. One potential explanation for resistance to targeted therapies may lie in the development of reversion mutations that restore DDR functions such

as HR. Such reversion mutations can restore the gene reading frame and remove a prior-existing deleterious alteration, and may be substitutions, insertions or deletions¹³⁴. For example, reversion mutations in *BRCA1/2* have been shown to partially restore WT gene function^{135,136}. Additionally, mutations and/or downregulation of other genes with roles in preventing HR may also restore HR activity in HRD tumours. For example, mutations in the genes encoding 53BP1 and REV7 and downregulated 53BP1 expression have been identified in PARPi resistant in *BRCA1* -/- breast tumours^{137–139}. 53BP1 functions along with Rif1 and Rev7 to recruit the shieldin complex to DSB ends, where it functions to prevent end resection and promote NHEJ mediated DSB repair^{140–143}. *BRCA1* functions to oppose this and has been shown to remodel 53BP1 away from DSB ends, thus presumably preventing shieldin binding and facilitating DNA end resection and HR. Indeed, loss of any of the key components of this pathway have been shown to restore DNA end resection and RAD51 loading in *BRCA1* deficient cells, suggesting that mutations and/or loss downregulated expression of any of these genes may play a role in drug resistance and HR restoration in HRD tumours and clouding the use of genomic scarring assays for HRD assessment and treatment selection.

It is possible that assays such as the DDIR assay, which are reflective of current, rather than historical tumour biology, may point the way forward, both in terms of identifying patients with an HRD likely to respond to DNA-damaging targeted therapies and which may also respond to immune checkpoint blockade due to upregulation of the DNA damage driven immune response described earlier. However, additional clinical validation of this assay is awaited.

In conclusion, HRD remains a key therapeutic target in breast cancer, and there is a pressing clinical need to develop robust, validated biomarkers identifying HRD tumours which will respond to targeted therapies. It is highly likely that the optimal setting for targeting HRD is

in the early disease setting (either neoadjuvant or adjuvant), potentially with combination therapies, to ensure maximal tumour cell eradication before resistance mechanisms, such as reversion mutations, have the opportunity to arise from the genomic disorder of advanced disease.

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Table 1: Mutated homologous recombination genes causing syndromes associated with hereditary breast cancer, and their estimated relative risks.

Gene	Function	Syndrome	RR	Reference
High-penetrance mutations				
<i>BRCA1</i>	Encodes a tumour suppressor, roles in maintaining genomic integrity.	Hereditary breast/ovarian cancer syndrome	>5	[31]
<i>BRCA2</i>	Maintenance of genomic integrity, specifically the HR pathway.	Hereditary breast/ovarian cancer syndrome	>5	[31]
Tumour protein p53 (<i>TP53</i>)	Encodes a tumour suppressor, controls cell cycle and activating DNA repair proteins.	Li-Fraumeni Syndrome	2–10	[36],[37]
Phosphatase and tensin homologue (<i>PTEN</i>)	Encodes a tumour suppressor phosphatase enzyme implicated in cell proliferation, survival and growth.	Cowden Syndrome	2–10	[38],[39]
BRCA1-partner and localiser of BRCA2 (<i>PALB2</i>)	Encodes a protein that interacts with BRCA2 and required for some functions of BRCA2 in HR.	<i>PALB2</i> -related	2–4/>5	[40],[41],[42]
Moderate-penetrance mutations				
Checkpoint kinase 2 (<i>CHEK2</i>)	Encodes a cell cycle checkpoint kinase and is an upstream regulator of BRCA1.	<i>CHEK2</i> -related	2–3	[43],[44]

Ataxia telangiectasia mutated (<i>ATM</i>)	Encodes a cell cycle checkpoint kinase and coordinates DNA repair.	<i>ATM</i> -related	2–3	[45],[46]
BRCA1 interacting protein C-terminal helicase 1 (<i>BRIP1</i>)	Encodes a nuclear protein that directly interacts with BRCA1, and is important to the function of BRCA1 in DSB repair.	N/A	2–3	[47],[48]

RR, Relative risk; N/A, non-applicable. Table adapted from Mavaddat and colleagues⁴⁹.

Table 2: Clinically relevant breast cancer subtypes and the reported association with HR gene mutations.

Breast cancer subtype	Receptor Expression	Description	Associated mutations
Luminal A	ER+/HER2-	<ul style="list-style-type: none"> Includes a number of low-grade variants Typically grow slowly Best prognosis⁶⁶ 	<i>BRCA2</i> <i>PALB2</i> <i>ATM</i> <i>CHEK2</i>
Luminal B	ER+/HER2+ Or ER+/HER2-/Ki67 high	<ul style="list-style-type: none"> Generally grow faster Worse prognosis than Luminal A Variable responses to therapies such as endocrine and chemo⁶⁶ 	<i>BRCA2</i> <i>PALB2</i> <i>ATM</i> <i>CHEK2</i> <i>TP53</i>
HER2-enriched	ER-/HER2+	<ul style="list-style-type: none"> High grade Grow faster than Luminal cancers Successfully treated with targeted therapies (e.g. trastuzumab)^{67–69} 	<i>BRCA2</i> <i>PALB2</i> <i>ATM</i> <i>CHEK2</i> <i>RAD51C</i> <i>RAD51D</i> <i>TP53</i>
Basal-like (TNBC)	ER-/PR-/HER2-	<ul style="list-style-type: none"> Do not respond to endocrine therapy or trastuzumab Poor prognosis 	<i>BRCA1</i> <i>RAD51D</i>

ER = Estrogen receptor; PR = Progesterone receptor; HER2 = Human epidermal growth factor receptor 2 receptor; TNBC = Triple negative breast cancer; +, positive for receptor expression; -, negative for receptor expression; Ki67, protein encoded by the *MKI67* gene, higher Ki67 is associated with higher grade and aggressive cancers.

Table 3: Clinical trials assessing the use of platinum-based therapies, focusing on those in the context of HRD in breast cancer.

Study/Trial	Study/Trial Design	Drug/Agent	Study Population	Mechanism of Action (HR)	Status
PRECOG 0105/Cisplatin-1 and Cisplatin-2 ¹⁰⁰	Phase II, single-arm, open label	Platinum salts	NEOADJ TNBC	HRD+ patients showed a higher pCR	Completed
GeparSixto ¹⁰¹	Phase II, randomised, open label	Carboplatin	NEOADJ TNBC & HER2+	HRD+ patients were reported to have a better prognosis compared with HRD- patients	Completed
TBCRC030 ¹⁰²	Phase II, randomised, open label	Cisplatin versus paclitaxel	NEOADJ TNBC	Preliminary findings: HRD+ patients in the Cisplatin group showed a slightly higher pCR than those in the Paclitaxel group	<i>Ongoing</i>
TNT ¹⁰³	Phase III, randomised, open label	Carboplatin versus docetaxel	Unselected, advanced TNBC	No significant difference was observed in HRD+ patient tumours between both treatment groups	Completed
SWOG S9313 ¹⁰⁴	Phase III, randomised, open label	Doxorubicin and cyclophosphamide	ADJ TNBC	HRD+ patients showed a better DFS	Completed

TNT, Triple Negative Breast Cancer Trial; TNBC, triple negative breast cancer; NEOADJ, neoadjuvant chemotherapy; HER2+, Human epidermal growth factor receptor 2 receptor positive; ADJ, adjuvant chemotherapy; HR, homologous recombination; HRD+, homologous recombination deficiency positive; HRD-, homologous recombination deficiency negative; pCR, pathological complete response; DFS, disease-free survival.

Table 4. Clinical trials assessing the use of PARP inhibitor therapies, focusing on those in the context of HRD in breast cancer.

Study/Trial	Study/Trial Design	Drug/Agent	Study Population	Mechanism of Action (HR)	Status
ICEBERG 1 ¹¹¹	Phase II, single-arm, open label	Olaparib (400mg bd versus 100mg bd)	Advanced, <i>BRCA</i> mutant BC (n=85)	HRD+ <i>BRCA</i> + patients in the ORR was higher in the cohort administered olaparib at the higher dose:	Completed
				<ul style="list-style-type: none">• ORR 41% vs 22% (high dose v low dose)• PFS 5.7 months vs 3.8 months (high dose vs low dose)	
OlympiAD ¹¹²	Phase III, randomised, open label	Olaparib versus physician chemotherapy choice	Pre-treated, advanced, <i>BRCA</i> BC (n=302)	<ul style="list-style-type: none">• ORR 59.9% vs 28.8%• PFS 7.0 vs 4.2 months (HR 0.58, p<0.001)• Possible to stratify patients based on HRD+ status	Ongoing
EMBRACA ¹¹³	Phase III, randomised, open label	Talazoparib versus physician chemotherapy choice	Pre-treated, advanced, <i>BRCA</i> BC (n=431)	<ul style="list-style-type: none">• ORR 62.6% vs 27.2%• PFS 8.6 vs 5.6 months (HR 0.54, p<0.001)• HR for death 0.76 at interim analysis	Completed

BROCADE 3 ¹¹⁴	Phase III randomised double-blind	Carboplatin/paclitaxel +/- veliparib	Pre-treated metastatic <i>gBRCA</i> mutant BC (n=513)	<ul style="list-style-type: none">PFS 14.5 months vs 12.6 months (HR 0.71, p=0.0016)	Completed
LUCY ¹¹⁵	Phase IIIb single arm open label study	Olaparib	Germine or somatic <i>BRCA</i> mutation, HER2 -ve breast cancer (n=250)	Ongoing – no results available	<i>Ongoing</i>
Olympia ¹¹⁶	Phase III randomised double-blind study	Adjuvant olaparib vs placebo (on completion of (neo)adjuvant anthracycline/taxane chemotherapy	<i>gBRCA</i> mutant high risk TNBC	Ongoing – no results available	<i>Ongoing</i>
NOBROLA ¹¹⁷	Phase IIa, non-randomised, open label	Olaparib	Non- <i>BRCA</i> TNBC (n=39)	<i>Ongoing</i>	<i>Ongoing</i>

RUBY ¹¹⁸	Phase II, single-arm, open label	Rucaparib – single agent	Non- <i>gBRCA</i> pre-treated advanced HER2 -ve BC (n=41), tumours with HRD as assessed by genome-wide LOH	13.5% of HRD+ patients showed benefit (as defined by LOH)	Completed (<i>whole genome sequencing ongoing</i>)
TBB ¹¹⁹	Phase II, non-randomised, open label	Talazoparib – single agent	Pre-treated, TNBC & HER2-, Non- <i>BRCA</i> BCs BUT had a germline HR gene mutation (n=20)	ORR 25% so some activity in advanced BC with a HR pathway mutation (dose reduction due to toxicity was required)	<i>Ongoing</i>
BrightTNess ¹²⁰	Phase III randomised	Paclitaxel vs paclitaxel + carboplatin vs paclitaxel +	Stage II-III TNBC (n=634)	<ul style="list-style-type: none">pCR 31% vs 58% vs 53%	Completed

		carboplatin + veliparib	Benefit from addition of carboplatin in unselected TNBC but no additional benefit from PARPi.	
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bd, twice daily; BC, breast cancer; TNBC, triple negative breast cancer; *gBRCA*, germline *BRCA* mutant; HER2-, Human epidermal growth factor receptor 2 receptor negative; HR, homologous recombination; HRD+, homologous recombination deficiency positive; ORR, objective response rate; PFS, progression-free survival, HR, hazard ratio; PARPi, PARP inhibitor.

Figure 1: The homologous recombination (HR) DNA repair pathway

The main HR proteins and mechanisms are illustrated. The MRN complex initially senses breaks in the DNA, in combination with the human single strand binding protein (hSSB1). The MRN complex is composed of meiotic recombination 11 (MRE11), the DNA repair protein RAD50 and Nijmegen breakage syndrome 1 (NBS1). MRN binds to the breakage site which both recruits and activates the checkpoint protein kinases ATM and CHK2, which in turn phosphorylate a number of substrates. BRCA1 associates with C-terminal binding protein interacting protein (CtIP), which then further interacts with the MRN complex. Then, DNA end resection begins by the nuclease MRE11, leaving 3' single-stranded DNA (ssDNA) overhangs at the DSB sites. These overhangs are coated with replication protein A (RPA) to form a nucleoprotein filament. RPA is displaced by partner and localiser of BRCA2 (PALB2) that recruits BRCA2, which loads the recombinase RAD51. This generates the Rad51-ssDNA-nucleoprotein filament that invades the homologous site on the double-stranded homologous sister chromatid leading to the error-free repair of DNA.

Figure 2: HR Deficiency (HRD) aetiology

The three types of HRD aetiology include epigenetic modifications (promoter methylation), structural rearrangements (LOH, TAI and LST – part of the ‘genomic scar’) and mutations (germline *BRCA*, germline HR genes and somatic HR mutations). These can lead to the inability of the HR pathway to repair damaged DNA and thus genomic instability as the damaged DNA is not efficiently resolved. Consequently, this can lead to the development of cancer, such as breast cancer development. g, germline; HR, homologous recombination; HRD homologous recombination deficiency; LOH, loss of heterozygosity; TAI, telomeric allelic imbalance; LST, large-scale state transitions.

Figure 3: Synthetic lethality in the context of HR and PARP inhibitor

The top boxed panel illustrates the normal circumstance whereby SSBs are repaired in the presence of PARP, during which the cell survives. When PARP is inhibited using a PARPi, SSBs are not repaired and in the case of some PARPis, PARP is trapped at the SSBs - both lead to replication fork stalling due to replication fork collision with the SSBs and/or trapped PARP. In HR proficient cells, the stalled replication forks are rescued by the HR proteins, which rescue the stalled fork via the HR pathway; as indicated in the left-arm of the diagram. Consequently, the cell survives. Contrary to this, as indicated in the right-arm of the diagram, in cells with an HRD, the stalled replication forks are not rescued leading to replication fork collapse and the accumulation of S-phase DSBs. These are not repaired in the context of an HRD, ultimately leading to cell death. In this case, the lack of HR to repair excessive damage caused by loss of PARP demonstrates synthetic lethality. SSBs, single-stranded breaks; HR, homologous recombination; HRD-, HR deficiency negative; HRD+, HR deficiency positive; PARPi, PARP inhibitor; DSBs, double-stranded breaks.

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