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Review

Homologous Recombination Defects and Mutations in DNA Damage Response (DDR) Genes Besides *BRCA1* and *BRCA2* as Breast Cancer Biomarkers for PARP Inhibitors and Other DDR Targeting Therapies

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Abstract. *Homologous recombination repair (HRR) is the cellular mechanism for error-free repair of double strand DNA (dsDNA) breaks. Cancer cells with mutations in both alleles of genes encoding for proteins involved in HRR, such as BRCA1 and BRCA2, have defects in the repair process. As a result, these cells repair dsDNA breaks with alternative mechanisms, such as non-homologous end joining. In breast cancers with germline mutations in BRCA1 and BRCA2 genes, HRR defects result in sensitivity to PARP inhibitors, drugs that interfere with the function of PARP enzyme and promote trapping of the enzyme on DNA and stalling of the process of repairing single strand breaks. HRR defects also lead to sensitivity to DNA damaging chemotherapy due to the inability of cells to repair chemotherapy induced DNA lesions. Besides germline mutations in BRCA1 and BRCA2, somatic mutations in these genes or germline and somatic mutations or other genetic and epigenetic alterations of other genes involved in homologous recombination (HR) may produce HRR defects leading to sensitivity to PARP inhibitors. However, studies are less conclusive, a fact that may relate to the common lack of bi-allelic loss of function in these cases, as opposed to cancers with germline BRCA1 or BRCA2 defects that usually acquire bi-allelic loss of function. In addition, there is heterogeneity between the different HRR genes and the severity of the resulting HRR defects, as measured by HR defect assays. This review article examines the landscape of HRR gene mutations in breast cancer and the possible therapeutic implications of HRR defects other than germline BRCA1 and BRCA2 mutations for targeted therapies. Identification of a wider range of breast cancers with HRR defects may expand the subset of patients that derive benefit from PARP inhibitors and other DDR-targeting drugs in the clinic.*

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Since the introduction of tamoxifen more than 45 years ago, the breast cancer therapeutics field was one of the first to incorporate targeted drugs and biomarkers in its armamentarium. Subsequently, estrogen receptor (ER) expression has been established as predictor of efficacy to endocrine therapy (1). Later, targeted therapies for the treatment of HER2-positive breast cancers have been introduced and have significantly improved outcomes of that breast cancer sub-set (2, 3). More recently, additional targeted therapies have been developed for ER-positive cancers, including CDK inhibitors, without further biomarker specifications inside the metastatic ER-positive group, and PI3K inhibitors for ER positive, *PIK3CA* mutated metastatic breast cancers (4-6). The sub-set of breast cancers that are negative for the ER, progesterone receptor (PR) and HER2 (triple-negative) had up until recently no targeted treatment options due to absence of a defining marker for targeting and heterogeneity within the group (7). However, this changed with the identification of *BRCA1* and *BRCA2* mutated breast cancers, many of which are triple-negative, as good targets for poly ADP-ribose polymerase (PARP) inhibitors, due to the synthetic lethality between *BRCA1* and *BRCA2* genes loss of function and PARP inhibition (8).

HER2-negative breast cancer patients with germline mutations in *BRCA1* or *BRCA2* genes derive benefit from therapy with the PARP inhibitor olaparib in the adjuvant and the metastatic setting (9, 10). Another PARP inhibitor talazoparib improved progression-free survival (PFS) but not overall survival (OS) of advanced breast cancer patients with germline mutations in *BRCA1* or *BRCA2* (11, 12). Both drugs have been approved for use in breast cancer patients with germline *BRCA1* or *BRCA2* mutations (13). Clinical development of two other PARP inhibitors, rucaparib and niraparib has resulted in regulatory approvals in ovarian cancer, but not in breast cancer yet (14, 15). Combination of PARP inhibitors with chemotherapy are also pursued, although overlapping toxicities exist (16). Another PARP inhibitor that may be more easily combined with chemotherapy due to a weaker PARP inhibition activity is veliparib (17, 18). Combination of veliparib with carboplatin and paclitaxel resulted in prolongation of PFS compared with chemotherapy alone in advanced HER2-negative breast cancer patients with deleterious germline *BRCA1* or *BRCA2* mutations (19). In contrast, results of a neo-adjuvant trial in triple-negative breast cancer showed that addition of veliparib to carboplatin and paclitaxel did not improve pathologic complete response or event-free survival outcomes (20, 21). Beyond germline *BRCA1* and *BRCA2* mutations, the role of other DDR-associated mutations or of somatic *BRCA1*/ *BRCA2* mutations as sensitizers to PARP inhibitors is less well determined. This article reviews the landscape of HR-associated gene mutations in genomic studies of breast cancer and will discuss the effectiveness of PARP inhibitors and other DNA damage response (DDR) inhibitors that are in clinical development in breast cancers with DDR defects. Data on breast cancers with somatic *BRCA1* and *BRCA2* mutations and with mutations of HR associated genes beyond *BRCA1* and *BRCA2* will also be reviewed.

Data Collection

The search for relevant articles and reviews was performed through the PubMed database of the United States National Library of Medicine (www.pubmed.ncbi.nlm.nih.gov). Search terms included "breast cancer", "homologous recombination", "DNA damage response", "PARP inhibitors", "olaparib" and "talazoparib". Retrieved articles were manually checked for relevance. References of relevant articles were also scanned for additional publications to be retrieved and included in the discussion.

Three large genomic breast cancer studies, the cancer genome atlas (TCGA) breast cancer study, the METABRIC study and the MSK metastatic breast cancer study, which are included in the cBioportal site (www.cbioportal.org) and provide data for molecular alterations in an extended panel of genes involved in DDR, were surveyed for alterations in core DDR genes (22-25). cBioportal is a cancer genomics site that harbors several pan-cancer and primary site-specific studies with individual patient level genomic data (25). Among the studies included in the evaluation of DDR associated genes, TCGA employs a next generation sequencing (NGS) whole exome genomic platform, while the two other studies use targeted sequencing platforms (22-24). The METABRIC study uses a targeted platform that includes 173 cancer associated genes and the MSK metastatic study uses the MSK-IMPACT platform that includes 341 to 468 genes.

DNA Damage Response and Repair of Double Strand Breaks

DNA damage is ongoing in living cells as a result of external insults, such as environmental chemicals and ionizing radiation or innate processes, such as nucleotide mismatches during replication (26). DDR describes the process of the recognition of a DNA lesion and the triggering of molecular events that result in repair of the abnormality. DDR machinery promotes inhibition of the cell cycle to ensure time for the repair and, in this manner, safeguard daughter cells from inheriting the DNA alteration (26). If DNA damage is irreparable, DDR machinery promotes permanent cell cycle arrest (senescence) or even triggers programmed cell death (apoptosis) (27). Different DNA lesions prompt alternative DDR pathways. For example, single strand DNA breaks are repaired by base excision repair, bulky adducts in the DNA are repaired by nucleotide excision repair and base mismatches are repaired by the mismatch repair pathway. Double strand breaks may be repaired by different mechanisms depending on the phase of the cell cycle and the existence of a complementary double strand (27). If a complementary double DNA strand is present, double strand breaks are repaired through HRR, using the intact helix as a template (28). This process is initiated by kinases ATM and ATR, which recognize the double break and recruit other proteins, such as BRCA1 and BRCA2, PALB2 and the RAD51 homologous proteins. BRCA1 is recruited first by ATM at the DNA damaged site and serves as the docking site for the MRN complex consisting of proteins MRE11, RAD50 and NBN, which create single strand extensions in the broken site by 5' end rejections. BRCA2 in co-operation with PALB2 help load RAD51 onto the single strand DNA projections, which become able to invade the sister strands and use it as a template for production of DNA extensions that are then ligated to repair the break (29). Kinase ATR, which has a primary role during replication at stalled forks, inhibits cyclin dependent kinases (CDKs) activity, initially required for DNA end resection, to promote cell cycle arrest (30). In the absence of a complementary strand, double strand breaks are repaired using alternative mechanisms, such as non-homologous end joining and alternative or microhomology-mediated end joining which, in contrast to homologous recombination, are

Table I. *Mutations in DDR-related genes in breast cancer from TCGA.*

Gene	Entire cohort		Luminal A		Luminal B		HER2+		TNBC	
	All mutations (n=1,066 profiled)	(Likely) oncogenic	All mutations (n=499 profiled)	(Likely) oncogenic	All mutations (n=197 profiled)	(Likely) oncogenic	All mutations (n=78 profiled)	(Likely) oncogenic	All mutations (n=171 profiled)	(Likely) oncogenic
BRCA1	27 (2.5%)	14 (1.3%)	9 (1.8%)	3 (0.6%)	3 (1.5%)	2 (1%)	2 (2.6%)	0	13 (8%)	9 (5.3%)
BRCA2	35 (3.3%)	14 (1.3%)	9 (1.8%)	4 (0.8%)	4 (2%)	2 (1%)	7 (9%)	1 (1.3%)	6 (3.5%)	2 (1.2%)
PALB2	7 (0.7%)	1 (0.1%)	2 (0.4%)	0	0		4 (5.2%)	1 (1.3%)	0	
RAD51	2 (0.2%)	0	0		2 (1%)	0	0		0	
RAD51B	3(0.3%)	1 (0.1%)	2 (0.4%)	1 (0.2%)	1 (0.5%)	0	0		0	
RAD51C	6 (0.6%)	2 (0.2%)	2 (0.4%)	0	3 (1.5%)	2 (1%)	1 (1.3%)	0	0	
RAD51D	2 (0.2%)	0	1 (0.2%)	0	0		1 (1.3%)	0	0	
RAD50	9 (0.9%)	1 (0.1%)	4 (0.8%)	1 (0.2%)	3 (1.5%)	0	1 (1.3%)	0	1 (0.6%)	0
XRCC2	3 (0.3%)	0	1 (0.2%)	0	0		0		2 (1.2%)	0
ATM	25(2.3%)	16 (1.5%)	9 (1.8%)	7 (1.4%)	9 (5%)	3 (1.5%)	2 (2.6%)	1 (1.3%)	4 (2.3%)	2 (1.2%)
ATR	14 (1.3%)	4 (0.4%)	4 (0.8%)	2 (0.4%)	3 (1.5%)	1 (0.5%)	4 (5.2%)	0	3 (1.8%)	1 (0.6%)
BRIP1	10 (0.9%)	1 (0.1%)	3 (0.6%)	0	4 (2%)	1 (0.5%)	3 (4%)	0	0	
NBN	7 (0.7%)	1 (0.1%)	4 (0.8%)	1 (0.2%)	0		0		3 (1.8%)	0
MRE11	5 (0.5%)	0	2 (0.4%)	0	2 (1%)	0	0		1 (0.6%)	0
CHEK1	4 (0.4%)	2 (0.2%)	2 (0.4%)	2 (0.4%)	0		2 (2.6%)	0	0	
CHEK2	9 (0.9%)	3 (0.3%)	4 (0.8%)	2 (0.4%)	1 (0.5%)	0	2 (2.6%)	1 (1.3%)	1 (0.6%)	0
BARD1	0		0		0		0		0	
WEE1	6 (0.6%)	0	3 (0.6%)	0	0		1 (1.3%)	0	2 (1.2%)	0
POLQ	16 (1.5%)	0	7 (1.4%)	0	2 (1%)	0	5 (6.5%)	0	2 (1.2%)	0
CDK12	13 (1.2%)	4 (0.4%)	4 (0.8%)	2 (0.4%)	5 (2.5%)	0	1 (1.3%)	0	2 (1.2%)	1 (0.6%)

The sum of the cases of the 4 subtypes do not always add up to the number of cases with mutations in the whole cohort as some cases had not been sub-typed. DDR: DNA damage response; TNBC: triple-negative breast cancer; TCGA: The Cancer Genome Atlas.

more prone to errors (31, 32). In cancers with *BRCA1*/*BRCA2* mutations or other defects in homologous recombination repair, cells are dependent on PARP enzyme to carry repair of their damaged DNA and are thus prone to apoptosis, if the enzyme is inhibited by PARP inhibitors, a concept known as synthetic lethality (8). PARP plays also a role in repair by microhomology-mediated end joining, which contributes to *BRCA1* and *BRCA2* defective cells exposed to PARP inhibitors becoming vulnerable to apoptosis (33). In contrast, cells with no *BRCA1* or *BRCA2* defects are much less sensitive to PARP inhibition. Polymerase theta, encoded by *POLQ* gene is the polymerase that fills the gaps during microhomology-mediated end joining and antagonizes homologous recombination by competing with RAD51 loading (34). Up-regulation of polymerase theta due to *POLQ* amplification or POLQ expression up-regulation, which is observed in cells with *TP53* mutations, such as triple-negative breast cancer cells, may lead to homologous recombination defects (35). Defects in transcription associated kinase CDK12, which, in conjunction with cyclin K, phosphorylates the carboxyterminal domain of RNA polymerase II and activates transcription, may also lead to homologous recombination dysfunction through down-regulation of genes involved in the process (36).

The Landscape of Breast Cancers With DDR-associated Mutations

Overall, 157 cases (14%) from the TCGA cohort have at least one mutation in DDR associated genes and 112 cases (10%) have at least one mutation in non-*BRCA1*/ *BRCA2* DDR associated genes. *BRCA1* and *BRCA2* mutations are observed overall in 27 cases (2.5%) and 35 cases (3.3%) of breast cancers, respectively (Table I). Other DDR genes, including *ATM*, *ATR*, *POLQ* and *CDK12*, are mutated in 1% or more breast cancer cases each and *RAD50*, *BRIP1*, *CHEK2* and others in lower numbers (Table I). Several of these mutations are likely or confirmed oncogenic, while other mutations are of unknown significance (Table I). Thirty patients (2.8%) have at least one likely or confirmed oncogenic mutation in non-*BRCA1*/ *BRCA2* DDR genes. The most commonly mutated DDR associated gene is *ATM*, mutated in 2.3% of cases, most of which (1.6%) are considered oncogenic or likely oncogenic. The HER2 sub-type possess the highest percentage of cases with non-BRCA DDR mutations (21%), followed by luminal B cancers (15%) and triple-negative cancers (12%). The lowest prevalence of cases with at least one non-*BRCA1*/ *BRCA2* DDR-associated gene mutation is observed in luminal A cancers at 9% (Table I).

In the METABRIC cohort, which included 2509 patients with mostly localized breast cancers, mutations in *BRCA1* and *BRCA2* were present in 1.7% and 2% of the whole cohort (Table II). About a third of these were oncogenic or likely oncogenic. The higher prevalence of these mutations was in the basal subtype (4.8% for *BRCA1* and 3.3% for *BRCA2*). Among other DDR associated genes that were included in the targeted genomic panel employed, *ATR* and *BRIP1* had a prevalence of more than 1% (3.7% and 1.1%, respectively). *ATR* mutations were present in 5.4% of HER2-positive cancers, in 5.3% of basal cancers and in 4.4% of luminal B cancers. Luminal B cancers had also BRIP1 mutations in 2.9% of cases.

In a recent study with 1,365 profiled metastatic breast cancers, mutations in *BRCA1* and *BRCA2* were present in 2.7% and 4.8% of cases respectively (24). Other DDR-associated genes commonly mutated in this cohort included *ATM* (4.2%), *ATR* (3.7%), *BRIP* (1.8%), *CDK12* (1.7%) and *PALB2* (1.5%). This study did not provide information on breast cancer sub-types.

Overall, these studies show that the genes for kinases ATM and ATR are the most prevalent DDR associated genes, besides *BRCA1* and *BRCA2*, in breast cancer, with a prevalence of 2.3% to 4.2% and of 1.3% to 3.7%, respectively. Other mutated genes with prevalence around 1% include *BRIP1*, *CDK12* and *PALB2*. Thus, these five mutated genes are of the outmost interest as targets of therapy.

Methods to Assess HR Defects

Genetic testing for germline mutations in *BRCA1* and *BRCA2* genes remains the most widely used method to detect homologous recombination defects in the clinic. Somatic mutations in *BRCA1* and *BRCA2* genes and other DDR associated genes can be assessed clinically on tumor material, although mutations in this category do not necessarily predict PARP inhibitors efficacy (see later). An alternative to checking for individual mutations in the machinery of HR is to use molecular signatures (genomic scars) resulting from recombination defects for determination of presence of an underlying defect agnostic to the specific causative molecular alteration(s) (37). These signatures are specific for the underlying defective process, such as homologous recombination defects but are observed as a result of various genetic or epigenetic alterations in genes involved in the process (38). Assays based on HR defect signatures have been developed and some of them have been validated and are in clinical use (39, 40). The HRDetect assay was developed using a lasso logistic regression model through identification of signatures predictive of the presence of *BRCA1* or *BRCA2* deficiency (39). The assay showed high sensitivity in identifying *BRCA1* and *BRCA2* deficient cancers in both breast and other cancer cohorts. Features

Table II. *Mutations in DDR-related genes in breast cancer from METABRIC (almost exclusively non-metastatic).*

Gene	Entire cohort		Luminal A		Luminal B		HER2+		Basal-like		Claudin-low	
	All mutations (n=2,509 profiled)	(Likely) oncogenic	All mutations (n=700 profiled)	(Likely) oncogenic	All mutations (n=475 profiled)	(Likely) oncogenic	All mutations (n=224 profiled)	(Likely) oncogenic	All mutations (n=209 profiled)	(Likely) oncogenic	All mutations (n=218 profiled)	(Likely) oncogenic
BRCA1	42 (1.7%)	16 (0.6%)	9 (1.3%)	2 (0.3%)	9 (1.9%)	2 (0.4%)	5 (2.2%)	1 (0.4%)	10 (4.8%)	8 (3.8%)	3 (1.4%)	2 (0.9%)
BRCA2	49 (2%)	16 (0.6%)	16 (2.3%)	4 (0.6%)	8 (1.7%)	2 (0.4%)	7 (3.1%)	1 (0.4%)	7 (3.3%)	4 (1.9%)	0	
ATR	94 (3.7%)	13(0.5%)	21 (3%)	4 (0.6%)	21 (4.4%)	2 (0.4%)	12 (5.4%)	4 (1.8%)	11 (5.3%)	1 (0.5%)	9 (4.1%)	0
BRIP1	29 (1.2%)	4 (0.2%)	5 (0.7%)	1 (0.1%)	14 (2.9%)	1 (0.2%)	0		2 (1%)	0	2 (0.9%)	2 (0.9%)
CHEK2	16 (0.6%)	6 (0.2%)	6 (0.9%)	1 (0.1%)	4 (0.8%)	3 (0.6%)	1 (0.4%)	0	0		1 (0.5%)	0

The sum of the cases of the molecular subtypes do not always add up to the number of cases with mutations in the whole cohort as some cases are not sub-typed. DDR: DNA damage response.

predicting *BRCA1* or *BRCA2* deficiency included micro-homology-mediated deletions, two substitution signatures, two rearrangement signatures and the HRD index (39). Using the HRDetect assay, 14% of ER positive breast cancers were shown to have HR deficiency, and thus be potentially appropriate for therapy with platinum chemotherapy or PARP inhibitors (41).

My choice HRD (Myriad Genetics Inc, Sault Lake City, UT, USA) is another genomic assay that calculates a HR defect score derived as the arithmetic mean of three partial scores depicting loss of heterozygosity, telomeric allelic imbalances and large-scale state transitions, respectively (42). The partial scores and even more strongly the combined HR defect score have been robustly correlating with the presence of *BRCA1* and *BRCA2* defects. The assay is concordant in patients' pre-chemotherapy and after chemotherapy treatment, suggesting that the HR deficiency is not affected by exposure to chemotherapy (43). FoundationFocus CDxBRCA (Foundation Medicine) is still another HR assay for use to guide PARP inhibitor use and comprises detection of germline and somatic mutations in *BRCA1* and *BRCA2* as well as determination of genome wide loss of heterozygosity (gLOH) (44, 45). RAD51 evaluation with immunohistochemistry (IHC) has also been used as a marker of HR deficiency (46). RAD51 IHC had a high concordance with the status of *BRCA1* and *BRCA2* genes and with the genomic HRD score from the My choice HRD assay in samples from the GeparSixto trial of triple-negative breast cancers (46).

Platinum and Other Chemotherapy Regimens in Breast Cancers With HR Defects

A theoretical interest for DNA damaging chemotherapy drugs in the treatment of HR defective cancers arise from the reduced ability of these cancers to repair defects induced by these drugs. Consistent with this assumption, in the sub-set of patients with HR defects in the Profiler-01 trial who received cisplatin, Disease Control Rate (DCR) was 80% in patients with *BRCA1* or *BRCA2* mutations, 55% in patients with other DDR gene mutations or *BRCA1* or *RAD51C* promoter methylations and 18% in patients without alterations (47). Patients with mutations in FANCL, FANCA and RAD51D and methylation in RAD51C were those among whom platinum treatment led to control of the disease. In an analysis of three neo-adjuvant trials of triple-negative breast cancer patients who received cisplatin with or without bevacizumab, or carboplatin with gemcitabine and iniparib, patients with a HRD score of >42 had a better response to therapy compared with patients with low HRD scores, independently of the presence of *BRCA1* or *BRCA2* mutations (48). HR deficient, as defined by an HRD score of >42, triple-negative breast cancer patients showed a benefit from addition of carboplatin to paclitaxel and liposomal doxorubicin in the GeparSixto trial, while patients that were HR proficient had no significant benefit from the addition of carboplatin (49). HR deficiency was present in 70% of patients in this study.

A meta-analysis of neo-adjuvant trials with or without platinum chemotherapy in triple-negative breast cancers showed a benefit for platinum containing regimens (50). However, in contrast to the BrighTNess trial and a retrospective series that showed benefit of platinum-based chemotherapy in germline *BRCA1* and *BRCA2* mutated patients (21, 51), the meta-analysis suggested that the benefit in pathologic complete response of neo-adjuvant platinum regimens was restricted to patients without *BRCA1*/ *BRCA2* mutations (50). Another meta-analysis that included both neo-adjuvant and adjuvant studies concluded for a benefit of platinum containing regimens in early triple-negative breast cancer (52). Part of these discrepancies undoubtedly stem from the heterogeneity of triple-negative cancers, which are in fact molecularly diverse (53).

Besides platinum-based chemotherapy, breast cancers with *BRCA1*/*BRCA2* mutations and HR deficiency, as measured by a HRD score of >42 in the My choice HRD assay, are shown to be more sensitive to neo-adjuvant chemotherapy with anthracyclines and taxanes, achieving significantly higher pathologic complete responses compared with patients who were wild type for *BRCA1*/ *BRCA2* and HR proficient (43). These data suggest that breast cancers with HR deficiency due to either *BRCA1*/ *BRCA2* deleterious mutations or due to other molecular alterations may be more sensitive to a wide array of DNA damaging chemotherapies compared to HR proficient cancers.

PARP Inhibitors in Breast Cancers With DDR Alterations Other than Germline BRCA1/BRCA2

PARP inhibitor olaparib is approved for use in HER2-negative metastatic breast cancers with deleterious or suspected deleterious germline B*RCA1* and *BRCA2* mutations (10). Talazoparib has also obtained approval for a similar indication in metastatic disease (12). In 2022, olaparib has been approved by the United States Food and Drug Administration for the adjuvant therapy of high risk early HER2-negative breast cancer patients with deleterious or suspected deleterious germline *BRCA1* and *BRCA2* mutations, who had received adjuvant or neo-adjuvant chemotherapy (9). Clinical data for use of PARP inhibitors in breast cancers with germline mutations of other HR related genes and in breast cancers with somatic mutations in these genes begin to accumulate but are more ambiguous and have not led to any approvals yet.

The TBCRC 048 trial enrolled two cohorts of metastatic breast cancer patients with non-germline *BRCA1*/ *BRCA2* HR related gene mutations who were treated with olaparib

(54). The first cohort included patients with germline mutations in genes other than *BRCA1* and *BRCA2* and the second cohort enrolled patients with somatic mutations in either *BRCA1*/ *BRCA2* or other HR related genes. Response rates in the two cohorts were 33% and 31%, respectively. Responses were observed in patients with somatic *BRCA1*/ *BRCA2* and germline *PALB2* mutations but not in any patients with *ATM* or *CHEK2* mutations or in any of the few isolated cases with *BARD1*, *RAD50*, *CDK12*, *BRIP1*, *BLM* and *FANCA* mutations (54).

A retrospective report on seven metastatic breast cancer patients who received off label olaparib showed that the four patients with somatic *BRCA1*/ *BRCA2* mutations achieved a partial response and had a median PFS of 6.5 months (55). The three patients with other mutations (one patient with somatic *ATM* mutation, one patient with germline *ATM* mutation and one with germline *BARD1* mutation) did not respond to olaparib and had a median PFS of 3 months (55). In unselected patients with triple-negative breast cancers receiving neo-adjuvant olaparib in the phase II PETREMAC trial, patients with germline mutations in *BRCA1*, *BRCA2* and *PALB2* genes showed responses to the drug (56). In contrast to the previous series, patients with somatic mutations in *BRCA1*, *ATRX*, *EMSY*, *MEN1*, *PTEN* and *SETD2* genes, that were associated with HR deficiency, had also higher rates of response compared to patients not carrying such mutations. In addition, *BRCA1* promoter methylations were associated with response.

A small phase II study of talazoparib in patients with HR gene mutations other than *BRCA1* and *BRCA2* included 13 patients with breast cancer (57). Four of the 13 patients (31%) had a partial response to talazoparib, and 6 additional patients had stable disease for a disease control rate of 77%. Clinical benefit rate (complete response + partial response + stable disease for at least 6 months) was 54% (57). All 4 patients with partial responses had germline mutations in *PALB2*. Patients with stable disease included additional cases with germline mutations in *PALB2*, a patient with germline *CHEK2* mutations (This patient with the longest duration of response had also a germline mutation in *FANCA* and a somatic mutation in *PTEN*), as well as patients with germline *BRIP1* mutations and somatic mutations in *ATM* and *ATR*. All 5 patients with germline mutations in *PALB2* assayed (1 patient had not material available for the test) showed a score above 33 in the My choice HRD test and 4 of 5 patients had a score above 42 (57). Of 6 patients with germline *PALB2* mutations, 3 patients had loss of heterozygosity in the locus and 2 additional patients had biallelic mutations.

In breast cancers with germline *PALB2* mutations, inactivation of the wild-type allele, either by somatic mutations or by loss of heterozygosity, was required for acquisition of defective HR, while germline *PALB2* associated breast cancers without biallelic inactivation did not present the signature of HR defects (58). In addition, a pan-cancer evaluation has shown that biallelic alterations of *PALB2* and other core HR genes but not monoallelic alterations were associated with genome wide loss of heterozygosity (45). A report on 2 prostate cancer patients with somatic pathogenic frameshift monoallelic *BRCA1* and *BRCA2* mutations in the context of Microsatellite instability showed that, in both cases, tumors were not sensitive to PARP inhibitors while they responded to immune checkpoint inhibitors (59). A pan-cancer analysis disclosed that *BRCA1* and *BRCA2* mutations were more common in tumors with microsatellite instability compared with microsatellite stable tumors, but they were in general monoallelic and not associated with genome wide loss of heterozygosity (59).

In breast cancers with germline *CHEK2* mutations, loss of heterozygosity of the wild type allele was observed in 48% of cases (60). However, even in cases with loss of heterozygosity producing loss of the wild type allele, HR repair defects as measured by a HRD score above 42 or the presence of defective HR repair-associated signature 3 were less prominent than defects observed in germline *BRCA1* and *BRCA2* associated cases. Only one of the 20 cases examined showed a HRD score above 42 (60). In another series examining breast cancers with germline *CHEK2* mutations, absence of defective HR repair-associated signature 3 suggested that these tumors are HR proficient (61). Lack of HR scars in cases with *CHEK2* mutations is consistent with preclinical breast cancer cell line studies that have shown absence of HR repair signatures in *CHEK2* mutant cells (62).

These clinical observations suggest that identification of reliable biomarkers of response to PARP inhibitors are critical for development of these drug in tumors with HR defects beyond germline *BRCA1*/ *BRCA2* mutations. Three biomarkers of resistance to neoadjuvant talazoparib in breast cancer patients with germline *BRCA1* and *BRCA2* mutations were loss of SHLD2, expression of a hypoxia signature and expression of a stem cell signature (63). Loss of SHLD2, a component of the shieldin complex protects double strand DNA breaks from end resection in a 53BP-dependent manner and promotes non-homology end joining (64). Consistently, loss of shieldin complex components in *BRCA1* null cells leads to PARP inhibitor resistance, attesting for the importance of non-homology end joining for PARP inhibitor efficacy (65). In the other hand, an analysis from the EMBRACA trial showed that *BRCA1*/ *BRCA2* loss of heterozygosity, identified in 82% of patients, DDR gene mutational burden and tumor HR deficiency assessed by global genomic loss of heterozygosity were not associated with talazoparib efficacy (66). As EMBRACA trial included patients with germline *BRCA1*/ *BRCA2* mutations, additional molecular markers, such as global scars may be less powerful predictors, as they inform on the previous HR defects in the tumor but are less apt to confirm the current status of ongoing HR deficiency.

A variation of the functional assay of HR deficiency measuring RAD51 foci formation by immunofluorescence was feasible in clinical formalin-fixed paraffin-embedded pathology samples and predicted PARP inhibitor sensitivity in human xenograft derived models (67, 68). The functional assay predicted PARP inhibitor sensitivity better than genetic assays based on HR related gene mutations and genomic assays based on signatures. Moreover, RAD51 foci assay predicted PARP inhibitor resistance in xenograft models and was concordant with resistance observed in the corresponding patients (69). In contrast, some patients and xenografts with absence of RAD51 foci formation remained sensitive to subsequent platinum chemotherapy treatment, suggesting that platinum chemotherapy is efficient in a subset of patients with PARP inhibitor resistance.

Biomarkers of response to PARP inhibitors not directly related to genes involved in HR or to scars produced by the repair defect have also been proposed. For example, proteasome unit PSMD4 amplification is associated with sensitivity to PARP inhibition and loss of amplification confers resistance to PARP inhibitors *in vitro* (70).

Other Inhibitors in Breast Cancers With DDR Alterations

The ataxia-telangiectasia mutated (*ATM*) gene, encoding for a kinase that belongs to the PI3K-related kinase family, plays a central role in DDR. Somatic mutations in *ATM* contribute to carcinogenesis by promoting genome instability whereas germline mutations predispose to familial breast cancer and are associated with HRD in *BRCA1*/ *BRCA2* wild type breast cancer (71). ATM-depletion can increase the sensitivity of breast cancer cells to PARP inhibitors *in vitro*, suggesting a potential therapeutic target (72). Currently, several ATM inhibitors are under development and are being evaluated in studies for solid tumors. AZD0156, a potent and selective oral ATM inhibitor was shown to be a strong radiosensitizer in preclinical studies of breast cancer cell lines (73). In this study, AZD0156 enhanced the response to olaparib in patient derived triple-negative breast cancer xenograft models by inhibiting the repair of olaparib-induced DNA damage (73). A phase 1 trial of AZD0156 as monotherapy or in combination with olaparib or FOLRIRI in patients with metastatic cancers was recently completed and results are pending (NCT02588105). AZD1390, another ATM inhibitor, has been shown to be a radiosensitizer in glioblastoma and lung cancer preclinical models (74). A synthetic lethal interaction between AZD1390 and an EZH2 inhibitor was identified in *BRCA1* mutated breast cancer cell lines (75). ATM inhibitor M4076 is also in phase 1 development (NCT04882917).

Inhibitors of the other kinase involved in DDR signaling, ATR exacerbate replication stress that is toxic to HR deficient cells (76). PTEN deficiency is synthetic lethal with ATR inhibition using inhibitor VE-821 in triple-negative breast cancers as these tumors, which are high grade, display high ATR levels, suggestive of reliance on the kinase to counteract proliferative stress (77, 78). The first ATR inhibitor tested in clinical trials, berzotinib (VX-970) did not show any clinical activity as monotherapy (79). However, a phase 1 trial combining berzotinib and cisplatin resulted in 15.4% partial response among the 26 included patients. One patient with triple-negative breast cancer among the 4 breast cancer patients included in the trial achieved a PR and recurred after 17 months (80). The combination of berzotinib and cisplatin was also evaluated in a phase 1b trial including 47 triple-negative breast cancer patients (81). The ORR was 23.4%, including CR in 4%, and an additional 38% of patients had SD. The median duration of response was 6 months and the median PFS was 4 months (81). However, an attempt to identify patient selection biomarkers was unsuccessful. A trial associating berzotinib with radiotherapy in chemotherapy resistant triple-negative breast cancer is ongoing (NCT04052555).

Combinations of the ATR inhibitor ceralasertib (AZD6738) and the PARP inhibitor olaparib have shown synergism in PARP inhibitor resistant pre-clinical models in the context of ATM deficiency (82). The mechanism of the synergism involves the ceralasertib-promoted release of cells from the G2 arrest induced by olaparib and stimulation of chromosomal instability (82). Ceralasertib in combination with olaparib showed an antitumor effect especially in *BRCA2* mutated triple-negative breast cancer patient-derived xenograft models (83). Interestingly, increasing the dose of either drug led to responses even in triple-negative breast cancer xenografts without *BRCA2* mutations. However, the phase 2 VIOLETTE trial (NCT03330847), which randomized 226 patients with metastatic triple-negative breast cancer treated in the second or third line between the combination of olaparib and ceralasertib versus olaparib alone, was terminated as no significant difference in ORR or PFS with the combination was observed. In addition, PFS was not improved with the combination in the *BRCA1*/ *BRCA2* mutated sub-group of the trial that included 83 patients. The lack of benefit may be related to the fact that the trial included a PARP inhibitor naïve population. The combination of ceralasertib and olaparib is also investigated in ovarian cancer (NCT03462342, the CAPRI trial).

Elimusertib (BAY1895344), a highly selective ATR inhibitor, demonstrated activity in a phase Ib trial against a range of advanced solid tumors with different putative deleterious DDR alterations (84). Among the 143 patients with advanced solid tumors included in the trial, 19 patients had HER2-negative breast cancer. Overall, the Clinical Benefit Rate of elimusertib was 35%. In patients with ATM loss, ORR was 8.9% and SD rate was 55.9% (84). Hematologic toxicity was the most frequent drug related adverse event. Combination studies are also ongoing,

investigating elimusertib with the PARP inhibitor niraparib and with the PD-1 inhibitor pembrolizumab. Several other ATR inhibitor trials are focusing on tumors with HR defects and replication stress. In the phase 1/2a TRESR trial, the ATR inhibitor RP-3500 in monotherapy showed molecular responses as determined by a circulating tumor DNA assay in 44% of the 55 evaluable patients (85).

Tyrosine kinase WEE1 serves as a critical component of the response to dsDNA breaks by phosphorylating CDC2, thereby activating the G2-M checkpoint and allowing the cells to repair the damaged DNA (86). Therefore, drugs that abrogate the G2-M checkpoint, such as WEE1 inhibitors may induce breast cancer cells death (87). Aberrant WEE1 expression has been observed in melanoma but also in other tumor types including breast cancer (88).

Advanced cancers with an increased level of genomic instability may require functional checkpoints to allow the repair of accumulating DNA lesions that accompany genomic instability. Therefore, WEE1 might be an attractive target in advanced tumors where its inhibition leads to irreparable DNA damage. WEE1 inhibition can increase replication stress by inducing aberrant firing of replication origins and depletion of the nucleotide pool (89). Preclinical studies suggest that WEE1 inhibition results in anticancer activity, both as monotherapy in certain biomarker-selected populations (such as *CCNE1* or *MYC* amplifications), or in combination with chemotherapy or radiation (90, 91). Clinical activity of adavosertib (AZD 1775), a highly potent inhibitor of WEE1 kinase, as monotherapy, was shown in recurrent uterine serous carcinoma with an ORR of 29.4% (92). A preclinical study demonstrated that adavosertib can enhance the sensitivity of triple-negative breast cancer cells to PARP inhibition with olaparib by diminishing the expression of the HRR proteins RAD51 and Mre11 (93). In another preclinical study, both ATR inhibitor ceralasertib and WEE1 inhibitor adavosertib have been shown in xenograft models to reverse resistance to olaparib (94). In the phase Ib STAR clinical trial, which included a few breast cancer patients, adavosertib, administered sequentially with olaparib, showed promising antitumor activity (95). Among the 13 patients enrolled in the trial, one of five breast cancer patients, who had a *BRCA2* mutation, obtained a partial response. Overall, three patients of the 12 evaluable patients in the trial had a partial response and 5 additional patients had stable disease lasting for more than 4 months. Common adverse effects were mild nausea, anemia, fatigue, vomiting and diarrhea (95). The sequential administration was chosen to avoid the excessive adverse effects observed with concomitant administration of the two drugs which is a significant barrier in the development of combinations that include WEE1 inhibitors.

DNA polymerase theta (POLQ) recently emerged as a new promising drug target for the treatment of HR-deficient tumors. POLQ expression is particularly high in subtypes of breast and ovarian tumors with defects in HR, where it mediates backup repair of dsDNA breaks, thus compensating for the loss of HR (96). As a result, POLQ is synthetic lethal with HR, and POLQ inhibition in HR-deficient tumors induces cell death. In addition, POLQ inhibition synergizes with PARP inhibitors in killing HR-deficient cells (96). POLQ is important for repair of dsDNA breaks through microhomology-mediated end joining (34). PARP enzyme is required for initiating the process and thus both PARP and POLQ inhibition affect successful repair through microhomology-mediated end joining. Inhibition of PARP interferes with recruitment of POLQ in dsDNA breaks. Given the observed additive cytotoxicity of POLQ inhibition and PARP inhibition have on HR-deficient cells, these data suggests that POLQ also may have functions outside the PARP-mediated microhomology-mediated end joining (MMEJ) (97). Indeed, the large molecule of POLQ possesses helicase and nuclease enzymatic activities, in addition to the polymerase activity (98).

The inhibitor of human POLQ Novobiocin binds purified POLQ protein and blocks its recruitment to sites of dsDNA damage, inhibiting MMEJ repair (99). Novobiocin selectively kills *BRCA1* and *BRCA2* deficient cells compared to wild-type cells and potentiates the cytotoxic effect of PARP inhibitors *in vitro* and *in vivo*. Moreover, novobiocin kills HR-deficient, PARP inhibitor resistant tumor cells. Accordingly, clinical trials have now been initiated for the use of novobiocin in the management of these tumors. Another POLQ inhibitor, ART4215 is currently being evaluated either as monotherapy or in combination with PARP inhibitors in advanced solid tumors (NCT04991480).

Conclusion

Targeted cancer therapies represent a milestone towards personalized treatment as they function *via* inhibition of cancer-specific alterations. Although PARP inhibitors deliver significant anti-tumor responses in cancers with defective HR, PARP inhibitor resistance is a common clinical phenomenon and limits the overall effectiveness of these agents. Building on combination PARP inhibitor strategies may be effective in a broader range of breast cancers with functional deficiencies in HR and the wider DNA damage response. Combinations of DNA damage response drugs with other targeted therapies may also be an avenue to explore. For example, PI3K inhibitors down-regulate BRCA1 and may sensitize tumor cells to PARP inhibition (100). A phase 1b trial of alpelisib and olaparib in heavily pretreated triple-negative breast cancers without germline *BRCA1* or *BRCA2* mutations showed a RR of 18% and disease control in 59% with a median duration of response of 7.4 months (101). PI3K inhibitor and PARP inhibitor combinations may also be candidates for exploration in breast cancer subsets depending on PI3K/ AKT signaling,

such as ER-positive/HER2-negative and AR-positive cancers (102). However, an additional consideration is the risk of adverse effects with combination therapies, and treatment dose and scheduling need to be optimized to maximize the overall risk-benefit ratio. Novel approaches, such as peptide drug conjugates that combine a peptide with a cytotoxic drug and allow the drug to target only cells in the acidic tumor pH, could aid in the development of combinations of cytotoxics with PARP inhibitors or other DDR inhibitors with an acceptable toxicity profile. For example, in a preclinical *in vivo* study using human xenografts with HR deficiency, ATR inhibitor ceralasertib displayed synergism with CBX-12, a peptide drug conjugate that combines a pH sensitive peptide with the potent topoisomerase I inhibitor exatecan (103). Another promising mode of treatment is immune cell therapy, which has shown preliminary efficacy in breast cancer (104). As adverse effects of this mode of therapy and DDR inhibitors may be non-overlapping, combinations could be feasible.

As clinical data from combination studies mature, and a better insight of patients deriving the greatest benefit is gained, second-generation trials of agents targeting DNA repair will be focusing on molecularly defined sub-sets. Identification of effective rational combinations will advance breast cancer therapeutics beyond the current metastatic treatment paradigm that relies on monotherapies (105). Targeting with rational combinations may further be assisted by *in silico* tools in development (106).

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

Ioannis A. Voutsadakis conceived the study; both Authors performed literature review, wrote, and revised the manuscript.

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