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Targeting DNA repair in breast cancer

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ABSTRACT

Targeting of DNA repair is an important therapeutic approach in breast cancer, particularly for *BRCA1/2* associated breast cancers and those characterized by a "BRCAness" phenotype including those with "triple negative" subtype. Various assays and scores have been developed to evaluate degree of homologous recombination deficiency in the hope that this would aid in predicting for susceptibility to DNA repair targeting agents, and yet, presence of a germline mutation in BRCA1/2 remains the strongest predictor for therapeutic efficacy of such agents. Pre-clinical studies suggested increased sensitivity to agents that damage DNA in a way that interferes with DNA replication forks and which subsequently require DNA repair by homologous recombination, such as platinum salts, and this data was further confirmed clinically. Recently published phase III data favor the use of PARP inhibitors amongst patients with *BRCA1/2* associated advanced breast cancer. Novel chemotherapeutic agents targeting DNA damage repair are under evaluation as well as further combinations of PARP inhibitors with immunotherapeutics and other biological agents.

1. Introduction

Amongst patients with breast cancer, therapeutic targeting of DNA repair has been most extensively studied in patients with hereditary mutations in *BRCA1/2* and to a lesser extent in triple negative breast cancers (TNBC), that lack expression of the estrogen and progesterone receptors and lack HER2/neu over-expression. Inherited mutations in *BRCA1/2* account for more than 90% of hereditary breast and ovarian cancers [1]. *BRCA1/2,* along with other genes in the Fanconi anemia (FA) pathway, play key roles in homologous recombination (HR), the main mechanism that repairs double-strand DNA breaks (DSBs) [2–4]. The failure to adequately protect the genome against endogenous and exogenous sources of DNA damage results in genomic instability and accumulation of oncogenic mutations. *BRCA* deficient tumors appear to be more sensitive to DNA-damaging agents such as alkylating agents or platinum salts, which generate double-strand DNA breaks (DSBs) [5,6]. Moreover, these HR-deficient (HRD) tumors are better responders to agents that target compensatory repair pathways in a synthetic lethal approach compared to HR-proficient tumors. The increased benefit of platinum salts and poly ADP-ribose polymerase 1 (PARP1) inhibitors (PARPi) is well-known in germline *BRCA1/2*-mutated tumors [7]. However, sporadic tumors with wild type BRCA may also display HR deficiency [8,9], leading to the use of PARPi and platinum salts beyond the *BRCA1/2* germline mutated tumors, but with more limited success. Because of the lack of well-defined biomarkers to characterize HRD status, prospective trials testing platinum salts and PARPi usually include a companion test with variable relevance.

In this review we focus on the development of drugs that target DNA repair beyond conventional chemotherapy in the context of HRD-breast cancer and the related concept of tumor *BRCAness*.

1.1. Phenotype of BRCA1/2 associated breast cancer

BRCA mutated breast cancers have inherent deficiency of HR DNA repair, displaying distinct clinico-pathological features as compared to sporadic breast cancers [10–12]. *BRCA1* related cancers are usually high grade, invasive ductal carcinomas with a higher incidence of medullary carcinoma histological subtype, lymphocytic infiltration, foci of necrosis and pushing margins. Between 60% and 90% of *BRCA1* tumors are estrogen receptor

Abbreviations: FA, Fanconi Anemia; HR, homologous recombination; DSBs, Double-strand DNA breaks; TNBC, Triple Negative Breast Cancer; DDR, DNA Damage Repair; PARP, poly-ADP ribose polymerase; PARPi, poly-ADP ribose polymerase inhibitors; HRD, homologous repair deficiency; LOH, Loss of heterozygosity; pCR, pathological complete response.

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negative, and fall into the "basal-like" subgroup of breast cancers [12–15]. Of note, it is suggested that they actually arise from luminal progenitors rather than from basal progenitor cells [16–18]. In addition, *BRCA1* associated breast cancers have higher incidence of p53 mutations [19,20], lack of PTEN expression [21], c-myc amplification and EGFR expression [10]. In contrast, breast tumors arising in BRCA2 mutation carriers do not differ from sporadic tumors with regard to ER and PR expression, and ER positivity was reported in the majority of these tumors [12,13]. It has been suggested that HER2 over-expression is uncommon in *BRCA1* and *BRCA2* carcinomas, with reported frequencies ranging from 0 to 3.7% [13,22]. However, in a comprehensive report of 3797 BRCA1 and 2392 BRCA2 associated breast cancers, HER2 positivity was reported in 10% and 13% respectively [12]. Both BRCA1 and BRCA2 associated tumors tend to be of high grade, with 77% and 50% of the tumors respectively being as classified grade III in the CIMBA consortium report [12]. Accordingly, it has been reported that ER positive tumors of *BRCA1* and *BRCA2* mutation carriers display significantly higher Oncotype Dx recurrence scores as compared to ER positive tumors in non-carriers, suggesting more aggressive phenotype [23,24]. Clinically, *BRCA1/2* associated breast cancers have demonstrated a higher response to chemotherapy both in the neoadjuvant [33–35] and metastatic settings [25,26], which is consistent with inherent deficiency of HR DNA repair.

1.2. Non-BRCA associated breast cancers with DNA-Repair deficiency and susceptibility

DNA-repair deficiency and susceptibility occur in non-TNBC, particularly in the setting of a germline mutation in other genes related to DNA repair pathways – for example PALB2 & CHEK2 breast cancers are often endocrine sensitive [27,28],nevertheless, the frequency of HRD is relatively low in endocrine sensitive and in HER2/neu over-expressing tumors [29].

TNBC have been shown repeatedly to be enriched for homologous recombination repair defects ([30–32]). Sporadic TNBC are sometimes grouped with *BRCA1*-mutated cancers. In addition to the phenotypic similarities between them, it was shown that TNBC have reduced function of *BRCA* proteins (often termed "BRCAness") [10]. Several mechanisms were reported that inhibit *BRCA1* in TNBC, including methylation of the *BRCA1* promoter [33], low *BRCA1* mRNA expression and high levels of ID4, a negative regulator of *BRCA1* [34]. It was also shown that TNBC were associated with defective DNA repair pathways [35,36]. Whether sporadic tumors with reduced *BRCA1* expression behave clinically like *BRCA1*-mutant tumors remains unresolved. Numerous studies have suggested that the presence of a germline *BRCA* mutation, somatic *BRCA* deficiency, significant homologous recombination deficiency (HRD) or genomic instability are associated with greater sensitivity to platinum agents [37–40].

2. Breast cancer associated with non-BRCA germline mutations in genes associated with the HR pathway

Mutated genes in the HR pathway that have been implicated in genomic instability and HR deficient breast and ovarian tumors include *BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK1, CHEK2, FAM175A, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D* and *CDH1* [41,42]. Pennington et al. found that 31% of 390 ovarian carcinomas had a deleterious germline (24%) and/or somatic (9%) mutation in one or more of the HR related genes, and the presence of germline and somatic HR mutations was highly predictive of primary platinum sensitivity and improved overall survival [42]. In the cancer genome atlas (TCGA) analysis, 9.3% of women with breast cancers had a germline mutation in *BRCA1, BRCA2, ATM, BRIP1, CHEK2, PTEN, NBS1 & RAD51C* and 20% of TNBC had either a germline or somatic mutation in *BRCA1* or *BRCA2* [30].

2.1. Biomarkers for HRD

Biomarkers that have been proposed for predicting HRD & BRCAness, include mRNA and miRNA signatures [43], "mutator phenotype" that identify HRD by looking at tumors' genome wide mutation signature reflecting defective DNA repair [44], DNA copy number alterations (CNA) [45], the HRD – LOH (loss of heterozygosity) score that scores LOH segments of intermediate size [46] and other tests that attempt to score genomic instability [47].

2.1.1. BRCA1 promoter methylation

The histopathologic and molecular characteristics of breast cancers with *BRCA1* promoter hypermethylation are similar to those of BC with *BRCA1* mutated breast cancers [48] mostly TNBC of high grade and association with medullary and mucinous subtypes [49] Other epigenetic defects in HR genes have been described [50]. However, in the TNT trial, that evaluated efficacy of platinum versus taxanes in advanced TNBC, BRCA1 promoter methylation did not predict for platinum sensitivity [26].

3. Tests for HRD (homologous recombination defect)

The tumor phenotype suggested by clinical and histopathologic features lacks sensitivity and specificity for predicting *BRCAness* and HRD, leading to intensive search for new, reliable molecular assays to identify tumors affected by HRD [47].

3.1. Composite scores

As there are no tests with high sensitivity or specificity for detecting HRD-tumors or for reliably predicting superior response to HRD directed therapy, composite scores combining several tests have been developed.

The MyChoice HRD test was developed by Myriad Genetics Inc (Utah, USA) and combined 3 HRD measures: LOH, TAI (telomeric allelic imbalance) and LST (large scale transition). This score was highly correlated with the presence of a *BRCA1/2* defect and loss of the second allele of the affected gene [51,52]. However, this test failed to predict for platinum sensitivity in the TNT trial [26].

The HR composite score was developed by Foundation Medicine and used *BRCA1/2* mutational status and percentage of LOH.

Notably, to-date none of these molecular assays, other than a germline mutation in BRCA, reliably identify tumors affected by HRD or predicts for clinical response to HRD directed therapies [6].

4. Therapeutic approaches for targeting DNA repair classic chemotherapeutic agents

Preclinical models demonstrated that *BRCA* mutant cells were more sensitive to chemotherapeutic agents that caused double strand breaks in DNA, such as platinum compounds, anthracyclines and alkylators [53–56]. Clinically, *BRCA1/2* associated breast cancers have demonstrated a higher response to chemotherapy both in the neoadjuvant [33–35] and metastatic settings [25,26].

Retrospective studies suggested diverse clinical responses of *BRCA1/2* mutated breast cancers to various chemotherapy drugs [57–59] with a greater response to platinum compounds [60,61] and less response to taxanes and CMF (cyclophosphamide/methotrexate/fluorouracil) [57,62–64]. In addition, multiple retrospective trials of neo-adjuvant therapy demonstrated a generally higher pathological complete response (pCR) rates in *BRCA1/2* associated breast cancers compared to sporadic tumors. Byrski et al. reported

pCR rate as high as 83% (10/12) in *BRCA1* carriers who received neo-adjuvant cis-platinum [60,63]. A neo-adjuvant study from MD Anderson reported higher pCR rates with anthracycline and taxane containing regimens in *BRCA1* mutated breast cancers. Thus, 26 of 57 *BRCA1* carriers (46%) achieved a pCR, compared to 3 of 23 *BRCA2* carriers (13%) and 53 of 237 BRCA non-carriers (22%) ($p = 0.0008$). Interestingly, *BRCA1* status predicted pCR independent of ER negativity, suggesting that it is not simply the association of *BRCA1* mutations with a particular intrinsic subtype that explains the sensitivity of these cancers to chemotherapy [65]. One study reported low pCR rate with neoadjuvant anthracyclines ± taxanes-based chemotherapy in only 3 of 29 (10%) *BRCA2*-mutant breast cancer patients compared to 13 of 67 (19%) sporadic ER positive patients, and suggested that *BRCA2* carriers patients are less responsive to neo-adjuvant chemotherapy [66]. Notably these studies included diverse molecular subtypes and treatment regimens and the number of *BRCA* mutation carriers were limited, $n = 44$ [63], $n = 102$ [60] and $n = 80$ [65]. A study on TNBC in which all patients received dose dense AC-T, demonstrated a significantly higher pCR amongst *BRCA1* carriers. However unlike the non-BRCA cases that had an excellent outcome if a pCR was achieved, those with a *BRCA1* mutation and a pCR did not have superior outcome to those with residual disease and a *BRCA1* mutation, suggesting that despite greater chemo-sensitivity amongst *BRCA1* carriers this did not necessarily translate into a survival benefit [67].

Retrospective studies including the study by Byrski et al. which reported a pCR rate of 83% (10/12) in *BRCA1* carriers who received neo-adjuvant cis-platinum [60,63] led to prospective studies evaluating the role of platinum agents in TNBC and *BRCA*-mutated populations. Favorable response of triple-negative breast cancers to platinum was demonstrated in the prospective neo-adjuvant study by Silver et al., with the two *BRCA1* mutated patients amongst those achieving a pCR [68]. The CALGB 40603 (Alliance) trial was a phase II study that randomized patients with stage II-III TNBC in a 2×2 factorial design to test the addition of either carboplatin or bevacizumab or both to standard neo-adjuvant chemotherapy of weekly paclitaxel $\times 12$ followed by 4 cycles of dose dense AC. The addition of carboplatin to weekly paclitaxel increased pCR in the breast and axilla to 54% as compared to 41%, $P = 0.0029$. A further update presented at SABCS in 2015 failed to demonstrate a survival advantage for the addition of carboplatin but the trial was underpowered to do so. However no data was presented as to the proportion of patients harboring *BRCA1/2* mutations. The Geparsixto was also a phase II study that randomized patients with stage II-III TNBC to receive neo-adjuvant therapy with paclitaxel and non-pegylated liposomal doxorubicin with or without carboplatin or bevacizumab or both. In this study 23% of the patients were <40 years and 17.2% harbored a *BRCA1/2* mutation. For those with a BRCA1/2 mutation, the pCR rate in the non-carboplatin arm was 66.7% compared with 36.4% in the non-*BRCA* patients. The addition of carboplatin increased the pCR in the non-*BRCA* patients to 55% but did not increase the pCR rate and improved DFS only for patients that did not have a *BRCA* mutation and not for the *BRCA*-mutated population [69,70]. It is worth noting that in this study the chemotherapy protocol did not include an alkylating agent. A recent review and meta-analysis of randomized trials investigating platinum-based versus platinum-free neoadjuvant chemotherapy in TNBC did not find increased pCR rates among the 96 BRCA-mutated patients included in two trials, in contrast to the *BRCA* wild type tumors [71]. Notably, definitive evidence for survival advantage from adding platinum to standard treatment is still lacking [35,58].

In the metastatic setting, it was found that hormone receptor negative BRCA1-associated disease was less responsive to taxane chemotherapy than sporadic HR negative disease while for hormone receptor-positive BRCA1-and BRCA2-associated disease sensitivity to taxane chemotherapy was similar to that of sporadic patients [64]. The TNT study compared first line chemotherapy with Carboplatin versus Docetaxel for metastatic TNBC. Among the 43 *BRCA1/2* mutation carriers, superior response rate and improved progression free survival were demonstrated for those who received Carboplatin while no significant difference between the chemotherapy regimens was demonstrated for the unselected 376 patients population. Of note, in this study the Myriad HRD score, BRCA1 mRNA-low and BRCA1 promoter methylation did not predict for platinum sensitivity [26]. A retrospective French study evaluated outcome for women with MBC and a *BRCA* mutation who received high dose chemotherapy & autologous stem cell transplantation between 2003 and 2012. The study included 235 patients of whom only 15 (6.4%) had a *BRCA* mutation. On multivariate analysis patients without a *BRCA* mutation had a worse prognosis with a HR of 3.08 (96%CI 1.1–8.6) compared to those with a *BRCA* mutation [72].

In view of the predictive value of a germline *BRCA1/2* mutation for response to platinum salts, this regimen should be considered in the treatment *of BRCA1/2*–mutated TNBC or endocrine-resistant advanced/metastatic BC previously treated with a taxane and an anthracycline, as suggested by the recent ABC guidelines [73].

4.1. Novel chemotherapeutic agents

4.1.1. Novel platinum agents – BTP-114

BTP-114 (Placon therapeutics) is a novel albumin binding cisplatin prodrug. A phase-1 study of the novel platinum agent BTP-114 (Placon therapeutics) for patients with solid organ tumors with either a BRCA mutation or DNA repair mutation or abnormal HRD test has completed recruitment (ClinicalTrials.gov identifier: NCT02950064).

4.1.2. Trabectedin/lurbinectedin

Trabectidin and Lurbinectidin (a trabectidin-analog) bind to the minor groove of DNA and amongst other activities interfere with the transcription-coupled nucleotide excision repair machinery to induce lethal DNA strand breaks.

Sensitivity to these agents is linked to efficient transcription-coupled nucleoside-excision-repair and deficient homologous recombination repair mechanism [74].

Trabectedin is a novel marine derived agent and has demonstrated promising activity in phase II studies in *BRCA*-deficient metastatic breast cancer [75,76] with an overall response rate of 17% in heavily pre-treated patients and 33% amongst the *BRCA2* carriers. Pre-clinical data suggests a synergistic effect between Trabectedin & Olaparib [77].

Lurbinectedin is a trabectedin analog. Encouraging phase II results presented in *BRCA1/2* deficient advanced breast cancer demonstrated an overall response rate of 41% in heavily pre-treated patients [78].

4.1.3. Sapacitabine

Sapacitabine is a novel nucleoside analog prodrug that induces single-stranded DNA breaks after incorporation into DNA resulting in an accumulation of double stranded DNA-breaks [79]. It is currently being evaluated in combination with Olaparib in patients with *BRCA* mutant advanced breast cancer (ClinicalTrials.gov Identifier: NCT03641755).

4.1.4. Novel topo-inhibitors

Sacituzumab govitecan-hziy (IMMU-132; Immunomedics) is an anti-Trop-2-SN-38 Antibody-Drug Conjugate that has topoisomerase I (Topo I)-inhibitory activity. SN38 which is an active metabolite

of irinotecan is coupled to the humanized anti-trophoblast cell-surface antigen 2 (Trop2) monoclonal antibody by a cleavable linker, the drug is preferentially delivered to cancer cells expressing Trop-2 and the SN38 is delivered both intracellularly and in the tumor microenvironment [80]. A phase1/2 study in heavily pre-treated metastatic TNBC patients demonstrated an objective response rate of 33% (95% CI 24.6,43.1) and a median duration of response of 7.7 months (95% CI 4.9,10.8) with myelotoxicity being the key adverse side effect [81,82].

Pre-clinical activity in combination with PARP inhibitors in TNBC (including both *BRCA*-associated and non-*BRCA* breast cancers) suggests the combination successfully exploits synthetic lethality [83].

5. Novel DNA damage repair agents

Novel DNA damage repair agents have been previously categorized into three key groups according to their mechanism of action [84,85] - agents targeting DDR sensor proteins – for example - PARP inhibitors (monotherapy or combinations), agents targeting DDR signaling proteins – for example – ATR inhibitors, ATM inhibitors and DNA dependent protein kinase (DNA-PK) inhibitors and agents causing transient cell-cycle delays – for example - checkpoint inhibitors, WEE1-inhibitors. Cell-cycle delays in combination with agents that interfere with DNA repair can result in synthetic lethality, replication fork arrest, accumulation of single strand breaks and ultimately in synergistic cell death. Additionally, there are agents that further attenuate inhibition of base excision repair. These agents are being evaluated either as monotherapy or in combination with radiotherapy, chemotherapy, PARP inhibitors and immunotherapy across solid organ tumors including breast cancer in early phase trials, each with promising pre-clinical activity. The most robust data is available for PARP inhibitors [84].

5.1. PARP inhibitors

Poly (ADP-ribose) polymerase1 (PARP1) and Poly (ADP-ribose) polymerase2 (PARP2) are members of the PARP family of nuclear enzymes and have been the key target in clinical therapeutics. Poly (ADP-ribose) polymerase1 (PARP1) plays a key role in the repair of DNA single-strand breaks through base excision repair. PARP inhibitors have several mechanisms of action including catalytic enzyme effect, “trapping” of protein on DNA, inhibition of replication fork progression and increasing double-stranded DNA breaks [86,87]. The potency of the different available PARP inhibitors are often characterized according to their ability to cause trapping, and this varies between the different agents. The inhibition of PARP1 leads to the accumulation of single-strand breaks in DNA and consequently to double-strand breaks at the replication forks. Normally, these double strand breaks are repaired by homologous-recombination (HR). However, when cancer cells deficient of HR due to absent *BRCA* are exposed to PARP1 inhibitors they accumulate unrepaired double-strand breaks that result in collapse of the replication forks and cell death. Such synergistic cell death resulting from concomitant inhibition of molecular pathways that are each dispensable when inactivated solely is a concept known as "synthetic lethality". Since the normal cells of *BRCA*-mutation carriers contain one functional allele of *BRCA*, they can still use HR and repair DSB, and therefore they are resistant to PARP inhibition. Thus, PARP inhibitors selectively target the cancer cells and are associated with relatively minor damage to the normal tissues [5]. While there were initial concerns about the long term effects of PARP inhibitors, specifically, concern about future risk of second malignancies such as leukemia, data from studies with longer term follow-up have been reassuring [88,89].

5.1.1. Olaparib

Olaparib is an orally available PARP inhibitor and was the first FDA approved PARP inhibitor both for germline *BRCA* mutated advanced ovarian cancer and advanced breast cancer. The pivotal trial assessing PARP inhibitors in a study population enriched for *BRCA* mutation carriers was published by Fong et al. [90]. This phase I trial of 60 patients, 22 of whom harbored known *BRCA* mutations, established the maximum tolerated dose (MTD) of olaparib at 400 mg twice daily. The most common reported side effects were grade 1–2 fatigue and mild gastrointestinal complaints and there was minimal haematological toxicity. Evidence of sustained anti-tumor activity was limited to patients with *BRCA*-associated cancers, of whom 63% experienced clinical benefit. A proof-of-concept study evaluating Olaparib in *BRCA*-associated advanced breast cancer was next published by Tutt et al. [91]. This was a phase II multicenter, multi-national study assessing two dosing schedules of olaparib: 100 mg or 400 mg twice daily. The study included 54 women who had received a median of 3 previous chemotherapy regimens. The objective response rate (ORR) was 41% (11/27) in the cohort receiving 400 mg twice daily and 22% (6/27) in the cohort on the lower dosing schedule. Stable disease was achieved in 44% of both cohorts. Most toxicities were low grade, the most common being fatigue, nausea, vomiting and anemia. Further phase II studies supported activity of olaparib amongst women with advanced breast cancer and a germline *BRCA* mutation [92,93]. The first Phase III study of Olaparib for advanced breast cancer was published in 2017. In the study by Robson et al., women were randomized 2:1 to receive either Olaparib 300 mg twice daily or treatment of physician choice (eribulin, capecitabine or vinorelbine) in this study patients had received prior anthracycline and taxane, those that were HR + had progressed on at least one line of endocrine therapy, and patients could not have relapsed within 12 months of neo/adjuvant platinum therapy or progressed during platinum therapy in the advanced setting. 205 women received Olaparib and 97 received standard therapy. Median age was 44 years (range 22–76). The olaparib arm had superior response rate, progression free survival and toxicity profile to the standard therapy arm, with a response rate of 59.9% compared with 28.8% and a median PFS of 7 months compared to 4.2months (HR 0.58, 96% CI0.43-0.8). Subgroup analyses suggested that benefit was most significant amongst those with triple negative disease and those with no previous platinum exposure [94]. Pre-planned subgroup analyses of overall survival demonstrated a survival benefit for those receiving olaparib in the first line setting with an overall survival of 22.6 vs 14.7 months, hazard ratio of 0.51 (95% CI 0.29–0.90) [95].

OlympiA is an international phase III randomized, placebo blinded study evaluating adjuvant olaparib in BRCA1/2 mutation carriers with triple negative breast cancer or high risk endocrine responsive breast cancer after completion of standard adjuvant chemotherapy and radiotherapy which has completed recruitment (ClinicalTrials.gov Identifier: NCT02032823).

Based on pre-clinical data that PARP inhibition would result in greater chemo-sensitivity, phase I & II studies combining olaparib with other chemotherapeutic agents (including, paclitaxel, cisplatin, carboplatin, cyclophosphamide & eribulin) [96–100] for patients with advanced solid organ tumors including breast cancer have been performed and published with promising results and are being further explored. Olaparib is being evaluated in the neo-adjuvant setting in combination with chemotherapy (ClinicalTrials.gov Identifier: NCT01042379). Olaparib has also been evaluated in combination with endocrine therapy [101]. Pre-clinical evidence suggesting that upregulation of the PI3K-AKT-mTOR pathway may result in PARP inhibitor resistance led to phase I study of Olaparib with the PI3K inhibitors, buparlisib [102] and

alpelisib [103] with promising early results.

Olaparib is also being evaluated in combination with radiotherapy for locally advanced breast cancer (ClinicalTrials.gov Identifier: NCT03598257).

5.1.2. Veliparib

Veliparib (ABT-888) is a potent orally available inhibitor of PARP-1 and PARP-2. Pre-clinical data demonstrated that veliparib potentiates the effects of DNA-damaging agents [104] and this under-scored the combination of veliparib with chemotherapy in clinical development.

The common side effects include nausea, fatigue and pancytopenia.

In a Phase I study combining veliparib with doxorubicin and cyclophosphamide, partial responses were seen exclusively in the 3/5 included *BRCA1/2* carriers, with no partial responses in the non-carriers [105]. In a trial combining veliparib with carboplatin in 22 patients with *BRCA*-associated metastatic breast cancer overall response rate was demonstrated in 67% of patients [106]. In a phase II trial assessing veliparib and temozolamide in 41 patients with metastatic breast cancer by Isakoff et al. [107], clinical activity was seen exclusively amongst the 8 *BRCA1/2* mutation carriers.

Phase I/II studies have been published or are ongoing evaluating combinations of veliparib with different chemotherapeutic agents including irinotecan, mitomycin, vinorelbine, metronomic cyclophosphamide, cisplatin, gemcitabine, eribulin, liposomal doxorubicin, carboplatin and paclitaxel. The BROCADE-2 study was a randomized Phase II study where patients with advanced *BRCA1/2*-associated breast cancer were randomized to paclitaxel and carboplatin with or without Veliparib. The overall response rate was 77.8% compared to 61.3% ($p = 0.027$) favoring the veliparib containing arm. There was a trend to improved PFS and OS that did not reach statistical significance favoring the veliparib containing arm. The addition of veliparib did not increase the toxicity of the chemotherapy. The study initially had an arm combining temozolamide and veliparib but this arm was ceased following a futility evaluation [108]. The combination is being further evaluated in the phase III BROCADE3 study (ClinicalTrials.gov Identifier: NCT02163694).

Veliparib is being evaluated in combination with Carboplatin in the I-SPY2 neo-adjuvant trial, an adaptive phase II study (ClinicalTrials.gov Identifier: NCT01042379). In this study *BRCA1/2* mutation carriers with TNBC subtype were significantly more likely to achieve a pCR than non-*BRCA* TNBC, with a predicted pCR of 75% compared with 29% [109] and gene-expression signature profiling that distinguished between *BRCA1*ness and non-*BRCA1*-like signatures, indicated greater response to the combination for the *BRCA1*ness [110].

In the Phase III BrighTNess study the addition of veliparib and carboplatin to paclitaxel followed by doxorubicin and cyclophosphamide was evaluated in the neo-adjuvant setting for women with triple negative breast cancer. There was no additional benefit from adding veliparib in addition to that achieved by adding carboplatin in obtaining pathological complete response, neither in the non-*BRCA* or *BRCA*-mutant patients [111]. However, a key limitation of this study is the low dose of veliparib, less than half of that used in the BROCADE II study, which may be a possible explanation for the limited impact of the addition of veliparib.

Veliparib is also being evaluated in combination with the epidermal growth factor receptor inhibitor Lapatinib in advanced triple negative breast cancer (Table 1).

Veliparib has been evaluated in a phase I setting in combination with radiotherapy for locally advanced breast cancer with significant local toxicity [112].

5.1.3. Rucaparib

Rucaparib is a potent PARP inhibitor available in both intravenous (IV) and oral formulation. Most common side effects include haematogical toxicity, nausea, vomiting, fatigue and diarrhoea. In a Phase II study evaluating rucaparib in *BRCA* mutation carriers with advanced ovarian and advanced breast cancer, two cohorts were included – one with intermittent IV dosing and one with continuous oral dosing. The study included 23 women with advanced breast cancer, the best response was stable disease 44% ($n = 8$) on the IV dosing and no responses in the 5 patients on the oral dosing [113]. Rucaparib has been evaluated in the phase I setting in combination with various chemotherapy regimens [114].

Rucaparib is being evaluated in combination with atezolizumab in advanced TNBC (Table 1).

Rucaparib is being evaluated in patients with TNBC with an incomplete response to neo-adjuvant chemotherapy in a number of combinations including with cisplatin (ClinicalTrials.gov Identifier: NCT01074970) and radiation therapy (ClinicalTrials.gov Identifier: NCT03542175).

There are currently no trials evaluating Rucaparib in a phase III setting in breast cancer.

5.1.4. Niraparib

Niraparib (MK-4827) is an orally available PARP inhibitor. A phase I study evaluating niraparib in solid organ tumors included 12 patients with advanced breast cancer. Common side effects include haemtological toxicity, fatigue, headache, abdominal pain, nausea & vomiting and anorexia. Two of the 4 patients with a *BRCA* mutation who had breast cancer had a partial response to treatment [115]. Phase III study results of the BRAVO trial comparing niraparib to physician's choice chemotherapy in *BRCA*-positive advanced breast cancer are awaited. Niraparib is being evaluated in combination with immunotherapeutic and other novel agents (Table 1).

5.1.5. Talazoparib

Talazoparib is considered one of the most potent PARP inhibitors. A Phase I study demonstrated a 50% response rate amongst *BRCA* mutation carriers with advanced breast cancer [116]. Common side effects include haematological toxicity, nausea, fatigue and diarrhoea. Results from the Phase II ABRAZO study presented by Turner et al. were presented at the 2017 annual meeting of the American Society of Clinical Oncologists. The trial evaluated two cohorts – platinum naive and platinum exposed. Talazoparib demonstrated impressive response rates in both cohorts, amongst both *BRCA1* and *BRCA2* carriers and amongst both triple negative and endocrine responsive subtypes. Results from the Phase III EMBRACA study comparing Talazoparib and chemotherapy (treatment of physician choice - capecitabine, eribulin, gemcitabine or vinorelbine) were presented at the San Antonio Breast Cancer Symposium in 2017. The study randomized 431 patients to treatment. The progression free survival was 8.6 months in the talazoparib as compared to 5.6 months in the chemotherapy arm, HR 0.54 (95% CI, 0.41, 0.71) [117]. Quality of life also favored the talazoparib arm [118]. Results from a single arm pilot study of neo-adjuvant monotherapy talazoparib for 6 months for patients with a germline mutation in *BRCA1/2* demonstrated impressive results with a 53% pathological complete response amongst the 18 evaluable patients [119]. There are ongoing studies evaluating Talazoparib in the neo-adjuvant setting (ClinicalTrials.gov Identifier: NCT01042379, NCT03499353) assnd in combination with chemotherapy in metastatic TN breast cancer (ClinicalTrials.gov Identifier: NCT02358200) as well as in combination with several novel agents (Table 1).

Table 1
Novel agents being evaluated in combination with PARP inhibitors in breast cancer or solid organ tumors.

PARP Inhibitor	Investigational agent		Study Phase	Estimated enrollment	Status	Clinical trials.gov identifiers
Olaparib	Immuno-therapeutic	Durvalumab	2	60	Recruiting	NCT03167619
		Durvalumab	1/2	25	Recruiting	NCT03594396
		Durvalumab	1	8	Recruiting	NCT03544125
		Durvalumab	1/2	384	Recruiting	NCT02484404
		Durvalumab	1/2	427	Recruiting	NCT02734004
		Atezolizumab	2	72	Recruiting	NCT02849496
	mTORC1/2 inhibitor	AZD2014	1	159	Active, not recruiting	NCT02208375
	AKT inhibitor	AZD5363	2	159	Active, not recruiting	NCT02208375
	VEGF inhibitor	Cediranib	1/2	162	Active, not recruiting	NCT01116648
		Cediranib	1/2	384	Recruiting	NCT02484404
		Cediranib	2	126	Recruiting	NCT02498613
	Heat-shock-protein-90 inhibitor	Onalesipib	1	40	Recruiting	NCT02898207
	ATR-inhibitor	Ceralasertib	1	250	Recruiting	NCT02264678
	MEK inhibitor	Selumetinib	1/2	90	Recruiting	NCT03162627
	HDAC inhibitor	Vorinostat	1	28	Not yet recruiting	NCT03742245
	Trabectidin analog	Lubrinectedin	1/2	100	Recruiting	NCT02684318
Veliparib	Epidermal growth factor receptor inhibitor	Lapatinib	Pilot	23	Active, not recruiting	NCT02158507
Rucaparib	Immuno-therapeutic	Atezolizumab	1	48	Recruiting	NCT03101280
Niraparib	Immuno-therapeutic	Pembrolizumab	1/2	121	Active, not recruiting	NCT02657889
	mTOR inhibitor	Everolimus	1	24	Recruiting	NCT03154281
Talazoparib	Immuno-therapeutic	Avelumab	1/2	242	Recruiting	NCT03330405
	Glutaminase inhibitor	Telaglenastat	1/2	92	Recruiting	NCT03875313

6. Resistance to PARP inhibitors

As demonstrated in the two published phase III studies [120,121], the clinical benefit from PARPi in metastatic *BRCA* mutant breast cancer is quite modest, with a median time to disease progression of 7 and 8.6 months, only 2–3 months longer that with traditional chemotherapy. Rapid development of resistance to these drugs is a critical problem in the clinic. Several resistance mechanisms have been proposed, the most validated of which are restoration of HR through secondary mutations within the *BRCA* genes that reactivate their function [122,123]. Other mechanisms that partially restore HR are inactivation of the p53-binding protein 1 (53BP1) [124] and loss of poly (ADPribose) glycohydrolase (PARG) that confers resistance of *BRCA2*-deficient tumor cells to PARP inhibition by restoring PAR formation, controlled DNA replication fork progression and the recruitment of downstream DNA repair factors [125]. Additional possible contributions to PARPi resistance are elevated levels of RAD51 [126], upregulation of enhancer of zeste homolog 2 (EZH2), which is the catalytic component of the polycomb repressive complex 2 (PRC2) [127] and point mutations in PARP-1 [128].

6.1. Overcoming PARP inhibitor resistance

Combining PARPi with other agents that inhibit HR (novel agents & chemotherapeutic agents), is a strategy aimed at both over-coming PARPi resistance but also at trying to sensitize HR-proficient tumors to PARPi [86]. Novel agents that target the DNA damage repair pathways and that are being evaluated in combination with PARPi include ATM (Ataxia Telangiectasia Mutated Protein) inhibitors, ATR (Ataxia Telangiectasia and Rad3-related Protein) inhibitors, Wee1 inhibitors, c-met inhibitors and PI3K inhibitors [115,129,130]. In pre-clinical models G-quadruplex interacting compounds have been found to be toxic to *BRCA1* and *BRCA2* deficient cells and olaparib-resistant *BRCA* deficient cells were found to be sensitive to these compounds and as such they are of interest for future clinical studies [115].

6.1.1. Immunotherapy

There is research that suggests that DNA damaging agents may have significant immune-modulatory effects that include: promotion of immunogenic cell death, changes in tumor microenvironment and stimulation of neo-antigen production that may each evoke immune response. *BRCA1/2* associated tumors are often characterized by lymphocytic infiltration and there is emerging data correlating response rate to immunotherapy with level of tumor-infiltrating lymphocytes.

The role of immunotherapy in breast cancer, particularly in tumors with DNA damage repair deficiency, is still under study, with the first phase 3 data of atezolizumab in combination with nab-paclitaxel in advanced triple negative breast cancer being published in 2018, demonstrating a suggested benefit in overall survival amongst those with PDL1 positive immune-cells [116].

7. Combinations of PARPi with immunotherapy

Combinations of PARPi with immunotherapies such as anti-CTLA4 and anti-PD1/PDL-1 are now being tested clinically. These studies are based on the hypothesis that BRCA-mutated tumors have a higher mutagenic burden and therefore potentially an elevated neo-antigen load, which may produce a stronger anti-tumor immune response [131,132], and on the finding that DNA double-strand breaks upregulates PD-L1 expression in cancer cells [133]. There is also evidence that BRCA deficiency may induce a STING (Stimulator of Interferon Genes) - dependent innate immune response [134], which might also influence the anti-tumor effect of PARPi/immunotherapy drug combinations. A phase II TOPACIO trial evaluating Niraparib + pembrolizumab in metastatic TNBC was presented at the 2018 ASCO meeting and showed promising durable clinical benefit both in patients with BRCA mutated and non-mutated tumors. Multiple combination trials with DDR and immune checkpoint inhibitors are ongoing [135].

7.1. Radiation therapy & targeting DNA repair

The BRCA proteins play a role in various processes of DNA damage response [3,136]. Thus, cells that lack BRCA protein activity and even cells that are heterozygous for BRCA mutations may have impaired ability to repair radiation induced DNA damage [137], and therefore may be more susceptible to radiation induced cell death, but also may be more vulnerable to radiation induced carcinogenesis [138–141]. Indeed, there are data supportive of both

mechanisms [138–141]. An increased risk of breast cancer was reported in BRCA carriers that were exposed to low dose ionizing radiation [142]. However, neither hypersensitivity nor increased toxicity was noted in BRCA mutation carriers when therapeutic doses of radiation were delivered to the breast [143–145] and no increase in subsequent malignancies was reported [146]. Moreover, in their report of 655 BRCA carrier patients, Pierce et al. found no added risk of contralateral breast cancer from scattered radiation at 10 and 15 years [144]. Potential reduction of breast cancer by prophylactic mammary irradiation was previously proposed [147] and subsequently supported by an experimental mouse model [148]. In mammary-cancer-prone mice, irradiation of the mammary glands on one side decreased the tumor incidence rate compared with the mammary glands on the contralateral shielded side [148]. In BRCA carrier patients, irradiation of the affected breast as part of breast conserving therapy significantly decreased the risk of subsequent cancer in that breast compared with the contralateral unaffected and non-irradiated breast [149]. Moreover, a recently published phase II non-randomized trial demonstrated that the addition of contralateral breast irradiation to *BRCA1/2*-associated breast cancer undergoing breast conserving therapy significantly reduced the risk of contralateral breast cancer at 5 years [150].

Possible explanations for the preventative effect of breast irradiation in BRCA mutation carriers are eradication of cells that already transformed, reduction of epithelial cell proliferation and cell division thereby decreasing the accumulation of genetic aberrations, and depletion of the luminal epithelial compartment which is the origin of *BRCA1/2*-associated breast cancers [151]. Radiation therapy in combination with novel agents that target DNA repair is currently under evaluation amongst patients with locally advanced breast cancer.

8. Conclusions

Therapeutic options for targeting DNA repair pathways are rapidly expanding. The role of targeting of DNA repair pathways is now well established amongst patients with advanced *BRCA1/2* associated breast cancer and there is emerging data amongst those with *BRCA1/2* associated early breast cancer. However, further study is needed to successfully and consistently identify biomarkers that will help identify which patients are particularly susceptible to DNA repair targeting agents – both for the TNBC population which are enriched for homologous recombination repair defects and for identifying the small subgroups of patients with HR-defects amongst the other breast cancer subtypes. Additionally, the role of DNA-repair targeting agents for those with breast cancer associated with germline mutations in other DNA-repair associated genes such as *CHEK2, ATM, PALB2 & NBN*, is yet to be established in the clinical setting, as to their role for those with somatic mutations in genes involved in DNA repair. The combination of DNA repair targeting agents with novel agents and immune-therapeutics is an exciting area of research that may further increase therapeutic efficacy through synthetic lethality, synergistic activity, immuno-modulation, overcoming resistance mechanisms and by sensitizing HR proficient tumors to DNA repair targeting agents.

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