Systemic Therapy for Hereditary Breast Cancers

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KEYWORDS

• Hereditary breast cancer • Chemotherapy • PARPi • Drug resistance • Biomarkers

KEY POINTS

- 5-10% of all breast cancers are associated with mutations in hereditary breast cancer genes several of which are involved in the DNA damage response.
- *BRCA1* and *BRCA2* mutation is not associated with poor prognosis in breast cancers, but infact better prognosis when treated with chemotherapy in comparison to similar non *BRCA* mutated breast cancers.
- Treatment with the small molecule PARP inhibitor olaparib after chemotherapy improves overall survival in patients with early breast cancer and germline *BRCA1* or *BRCA2* mutations.
- Platinum based chemotherapy is highly active in those with germline *BRCA1* or *BRCA2* mutations but cross resistance between platinum agents and PARP inhibitors can be mediated by somatic "reversion mutations" in *BRCA1* or *BRCA2* and is a clinical challenge.
- Understanding the underlying mechanisms of overlapping or distinctive resistance is vital to therapy development in this evolving area of translational medicine.

INTRODUCTION

Approximately 5% to 10% of the 2.3 million breast cancer cases diagnosed annually are associated with a mutation in a known hereditary breast cancer predisposition gene such as *BRCA1* or *BRCA2.*[1,2] The integration of genomics into the standard diagnostic pathways for breast cancer patients and the availability of targeted treatment approaches for those with hereditary breast cancer predisposition gene

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mutations means that the management of these patients is now distinct to that a decade ago. In this review, we discuss the recent advances made in systemic treatments for hereditary breast cancer and highlight future challenges that must be addressed for improvements in clinical outcomes to be achieved in this distinct subgroup.

Hereditary Breast Cancer Genes

Many of the germline mutations associated with hereditary breast cancer occur in "caretaker" tumor suppressor genes (genes whose normal function is to maintain the integrity of the genome and whose dysfunction leads to genome instability) and include *BRCA1, BRCA2, PALB2, ATM, CHEK2*, and *p53*.[3] For example, germline deleterious mutations in *BRCA1* (*gBRCA1m*) or *BRCA2* (*gBRCA2m)*, which play key roles in DNA repair by homologous recombination (HR), are associated with an increased lifetime risk of developing breast, ovarian, prostate and pancreatic cancer.[2,4,5] *gBRCA1m* carriers have a cumulative lifetime breast cancer risk of 46% to 60% and when diagnosed with breast cancer typically have the basal-like, triple negative (B-L, TNBC), subtype of the disease. *gBRCA2m* carriers have a cumulative lifetime breast cancer risk of 43% to 55% and tend to develop estrogen receptor-positive (ER+), luminal B subtype, breast cancers (significantly more so than for *gBRCA1m* carriers).[6–9] *gBRCA1/2m* breast cancers in those who have a strong family history are usually detected at a younger age than sporadic breast cancers.

Germline pathogenic variants in *PALB2 (Partner and localizer of BRCA2)*—also involved in DNA repair by HR—were first associated with increased cancer risk in 2007.[10] In 2014 Antoniou *and colleagues* reported a cumulative risk of developing breast cancer by 70 years old of 35% in patients with *gPALB2m.* This particular study included 311 women with *gPALB2m* of whom 229 had breast cancer, and 51 men of whom seven had breast cancer.[11]

BRCA1, BRCA2, and *PALB2* are often regarded as high-penetrance breast cancer susceptibility genes, penetrance here being defined as the likelihood of a particular genotype (eg, *gBRCA1m*) resulting in a related phenotype (breast cancer). The *TP53* tumor suppressor (encoding p53) is also a high penetrance hereditary breast cancer susceptibility gene; individuals who carry a germline mutation in *TP53* carry an 80% risk of developing breast cancer by 60 years old.[12] *TP53* has both caretaker (preventing genome instability) and gatekeeper (preventing uncontrolled cell division and the transmission of mutations to daughter cells) functions. Lower penetrance breast cancer susceptibility genes exist, including the caretaker genes *ATM* (*Ataxia telangiectasia mutant)* and *CHEK2* (*Checkpoint kinase 2).* The lifetime risk of developing breast cancer if an individual has germline deleterious mutations in either *ATM* or *CHEK2* is 25% to 30%.[13,14]

Homologous Recombination

BRCA1, BRCA2, and *PALB2* encode proteins involved in the DNA damage response (DDR), playing integral roles in the process of HR. HR, when using an available sister chromatid as a DNA repair template, is a highly conserved, error-free DDR pathway that is activated by the detection of double-stranded DNA breaks (DSB) and stalled DNA replication forks. Upon recognition of such DNA damage, the checkpoint kinase ATM is activated, which leads to a cascade of protein phosphorylation events that localize BRCA1 to the site of DNA damage. BRCA1 recruits the MRN complex (MRE11-RAD50-NBN) to the site of damage, which in turn resects DNA on either side of DSB, generating DNA with '3 single-stranded overhangs which becomes bound by the RPA protein. In a PALB2- and BRCA1-dependent process, BRCA2 loads

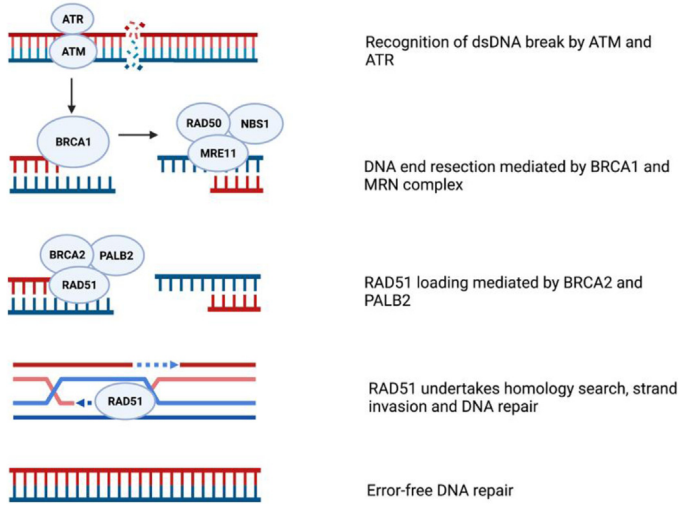

Fig. 1. Homologous recombination. On recognition of DNA damage, the checkpoint kinases ATM, and ATR are activated; BRCA1 localizes to the double-strand break and activates resection of the DNA through recruitment of the MRN complex, which generates 3′ overhangs. In a PALB2 and BRCA1-dependent process BRCA2 loads RAD51 onto single-stranded DNA at the double-strand break site. RAD51 then undertakes a homology search, strand invasion, and DNA repair. ATM, ataxia telangietasia mutant; ATR, ataxia telangietasia and RAD3 related.

the DNA recombinase RAD51 onto these 3′ single-stranded overhangs, displacing RPA. The DNA/RAD51 nucleoprotein filament that forms then uses RAD51's ATPase activity to invade the double helix of the homologous strand of DNA which is then used as a template for DNA synthesis, resulting in error-free DNA repair (**Fig. 1**).[15] Loss of function mutations in *BRCA1*, *BRCA2,* or *PALB2* cause a defect in this process, which ultimately leads to the elevated use of nonconservative forms of DNA repair. These nonconservative DNA repair pathways likely foster tumorigenesis by causing mutations in additional cancer driver genes (reviewed in Lieber *and colleagues*[16]).

Preclinical Evidence of Effects of BRCA1/2 Mutation on Chemotherapy Efficacy

Tumor cells with defects in HR, including those with *BRCA1* or *BRCA2* mutations, exhibit *in vitro* (and in some cases *in vivo*) sensitivity to drugs that cause forms of DNA damage that ultimately stall and/or collapse replication forks; these include mitomycin C,[17] bifunctional alkylating agents such as melphalan or the cyclophosphamide metabolite, phosphoramide mustard,[18] topoisomerase II inhibitors,[19,20] the DNA minor groove binding compounds lurbinectedin and trabectedin,[21] topoisomerases I inhibitors[22] and platinum-based chemotherapeutic agents.[23] There are five platinum chemotherapy analogs approved for the treatment of cancer: cisplatin, carboplatin, oxaliplatin, nedaplatin, and lobaplatin. The cytotoxic action of the two most commonly used in the treatment of breast cancer, cisplatin, and carboplatin, is largely caused by the formation of intrastrand DNA cross-links between purine bases—that is, platinum-containing molecular bonds between bases on the same DNA strand (interstrand cross-links being formed between bases on opposite strands). The distorted double helix formed by these cross-links is repaired by pathways including nucleotide

excision repair and HR. Given DNA damage induced by platinum chemotherapy relies heavily on the repair by HR, it is unsurprising that this group of patients typically respond well to platinum agents, and as such are now commonly given this in the neoadjuvant and advanced settings.[23]

Poly-(ADP Ribose) Polymerase Enzymes and Synthetic Lethality

PARP (Poly-(ADP Ribose) Polymerase) enzymes use NAD + to synthesize, poly-(ADP-ribose) (PAR) chains on substrate proteins, a process known as PARylation.[24] Most of the PARylation events that occur in cells are carried out by PARP1, a protein that detects damaged DNA including alkylated bases and single double-strand breaks in the double helix. PARP1 binds to DNA via N-terminal zinc-finger (ZnF) domains,[25,26] an event which causes a conformation change in PARP1's structure and activation of its catalytic activity. In broad terms, PARP1's PARylation activity instigates DNA repair by two mechanisms; by PARylating histones, PARP1 activity leads to the remodeling of chromatin structure to a degree that DNA repair is enabled, and by PARylating DNA repair proteins (including XRCC1) PARP1 activity concentrates DNA repair effectors at the site of DNA damage.[27] Once DNA has been successfully repaired, PARP1 auto-PARylates. This autoPARylation imparts a negative charge on PARP1, repelling it from DNA once it has detected and amplified the DDR.[28,29]

The role of PARP1 (and also its paralog PARP2) in DNA repair provided the rationale for discovering small molecule inhibitors of PARP1 and PARP2 (PARPi), which were initially envisaged to be used as chemo- or radiosensitisers.[30] Clinically approved PARPi such as olaparib, talazoparib, niraparib, and rucaparib trap PARP1 and 2 on DNA as well as inhibiting PARP catalytic activity; this PARP1 trapping capacity appears to make a greater contribution to tumor cell cytotoxicity in *BRCA1/2* defective tumor cells than the ability to inhibit the catalytic activity of PARP1. For example, the experimental PARPi veliparib is a potent catalytic inhibitor but has lower PARP1 trapping capacity when compared with other PARPi; its ability to elicit *BRCA1/2* synthetic lethality is also much reduced when compared with PARPi with higher trapping properties.[31,32]

In 2005, two independent research groups showed that *BRCA1* and *BRCA2* defective cells were profoundly sensitive to drug-like PARPi, both *in vitro* and *in vivo*.[33,34] Subsequent work showed that defects in other HR genes also caused profound PARPi sensitivity.[35] These observations provided the preclinical rationale for instigating clinical trials assessing the potential of PARPi as single agent synthetic lethal treatments for HR defective cancers, using *BRCA1m* or *BRCA2m* as a surrogate of this HR defective status and as patient stratification biomarkers.

Systemic Treatment in Early Breast Cancer

Neoadjuvant chemotherapy

BRCA1/2 mutation-associated breast cancer carries a better prognosis when treated with chemotherapy. For example, in a study conducted by Rennert *and colleagues* in 2007, the hazard ratio (HR) for death among *gBRCA1m* breast cancer patients receiving chemotherapy was 0.48 (95%CI 0.19–1.21, $P = .12$) compared with those who did not receive chemotherapy.[36]

Multiple studies have reviewed the efficacy of neoadjuvant chemotherapy (NACT) in *gBRCAm* breast cancer, some of which are summarized in **Table 1**. The surrogate endpoint for clinical efficacy in such studies is usually pathologic response, a metric measured by analyzing the residual disease volume in a surgical specimen retrieved following NACT. A prospective cohort study carried out by Byrski *and colleagues* in 2010 looked specifically at pathologic response in patients with *gBRCA1m* following

Table 1
Clinical trials published in early breast cancer

Trial Name	Phase	Treatment	Setting	Endpoint	Key Results	Authors
GeparSixto	II	Carboplatin vs SOC chemotherapy	Neoadjuvant	pCR	pCR 65 % vs 66.7% (*gBRCAm* group)	Hahnen et al.[41]
INFORM	II	Cisplatin vs AC	Neoadjuvant	pCR	pCR 18% vs 26%	Tung et al.[42]
ISPY-2	III	Veliparib + carboplatin (VC) + paclitaxel + AC vs paclitaxel + AC	Neoadjuvant	pCR	pCR 51% (VC TNBC group) vs 26% (no VC TNBC group)	Rugo et al.[47]
BrighTNess	III	3 groups: 1. Veliparib, carboplatin + paclitaxel (VCP) + AC 2. carboplatin + paclitaxel (CP) + AC 3. Paclitaxel + AC (P)	Neoadjuvant	pCR	pCR VCP 53% vs P 31% $P<.0001$. pCR VC 58%, $P = .36$	Loibl et al.[48]
GeparOLA	II	Olaparib + paclitaxel (OP) vs carboplatin + paclitaxel (CP)	Neoadjuvant	pCR	pCR OP 55.1% vs CP 48.6%	Fasching et al.[52]
OlympIA	III	Olaparib vs placebo	Adjuvant	IDFS	IDFS 87.5% vs 80.4%	Tutt et al.[53]
CREATE-X	III	Capecitabine + standard post-surgical treatment vs no capecitabine (control)	Adjuvant	DFS	DFS 69.8% vs 56.1%	Masuda et al.[59]

Abbreviations: AC, doxorubicin + cyclophosphamide; DFS, disease free survival; IDFS, invasive disease free survival; pCR, pathologic complete response; SOC, standard of care.

NACT (n = 103). They reported a higher pathologic complete response (pCR) rate (83.3%) in the group treated with cisplatin in comparison to other standard NACT regimens.[37] In the same year Silver *and colleagues* published data on the efficacy of neoadjuvant cisplatin in TNBC in a cohort of 28 patients, five of whom exhibited low tumoral expression of *BRCA1* mRNA and two of which had *gBRCA1m*.[38] The *gBRCA1m* carriers achieved pCR after treatment with cisplatin and the five patients with low *BRCA1* mRNA expression had a better clinical response to treatment than those with high *BRCA1* mRNA expression (although did not achieve pCR), suggesting that TNBC patients whose tumors exhibited "BRCAness" (ie, a phenocopy of *gBRCAm* cancer, for example, caused by reduced *BRCA1* mRNA levels)[39] may be more sensitive to platinum agents than non *BRCA* associated TNBC.

Data on germline *BRCA1* and *BRCA2* carriers have since become available from larger scale randomized control trials (RCTs) such as GeparSixto, a phase II RCT which reviewed the efficacy of adding carboplatin to standard of care (SOC) NACT. GeparSixto enrolled 588 patients with TNBC or HER2+ breast cancer who were randomized to receive either a backbone of paclitaxel and liposomal doxorubicin, or paclitaxel/liposomal doxorubicin plus carboplatin. An increased response rate was seen in the TNBC group subgroup who received carboplatin.[40] In a subsequent analysis, the same investigators sought to determine if *gBRCA1m* or *gBRCA2m* status affected therapy response in patients with TNBC. The study recruited 291 patients with TNBC, of which 50 had *gBRCA1/2m*. In the non-carboplatin arm, those with *gBRCAm* achieved a pCR rate of 66.7% (16/24 patients) in comparison to the non-*gBRCAm* group pCR rate = 36.4% (44/121 patients). The pCR rate in the carboplatin arm in those with *gBRCAm* was 65.4% (17/26 patients), but in those without *gBRCAm*, was 55% (66/120 patients). The investigators concluded that: (i) the addition of carboplatin benefitted the non-*gBRCAm* group; (ii) those with *gBRCAm* showed superior response rates to both carboplatin and non-carboplatin containing regimens; (iii) compared with those without mutations, the *gBRCAm* group did not exhibit any observed extra benefit from the addition of carboplatin.[41]

The INFORM study, a phase III RCT carried out by Tung *and colleagues*, assessed pathologic response in patients with stage 2 to 3 HER2– breast cancer with *gBRCAm*. Patients were randomized to receive single agent cisplatin (CDDP) or AC (doxorubicin + cyclophosphamide) before definitive surgery. The pCR rate in the CDDP group was 18% versus 26% in the AC group (risk ratio (RR) 0.70; 90% confidence interval (CI) 0.39–1.2). The investigators concluded that pCR was not significantly higher with CDDP versus AC in *gBRCAm* carriers.[42]

Data from GeparSixto and INFORM suggest that the addition of platinum treatment to the SOC NACT regimens does not necessarily improve the pCR rate of *gBRCAm* carriers. Tumors in this group of patients are sensitive to chemotherapeutic agents that intercalate or cross-link DNA such as anthracyclines or alkylating agents; this likely means that, when given in the neoadjuvant setting, the response to SOC chemotherapy is so high that pathologic response rates reach a plateau and any additional effect of platinum agents is thus modest. In contrast, when a DNA cross-linking platinum agent is directly compared with standard-of-care microtubule stabilizing agents in metastatic disease, *gBRCAm* does predict a greater response to the platinum (discussed later).[23,43]

Neoadjuvant poly-(ADP ribose) polymerase inhibitor monotherapy

The efficacy and safety of olaparib (PARPi) monotherapy preceding surgery was reported following a window of opportunity study carried out in 2013, where patients were randomized to receive an escalating dose of olaparib 4 days before surgery.[44]

A dose-dependent increase in exposure to olaparib was seen but at significantly lower plasma exposure levels than observed in advanced disease studies. The mean maximal PARP inhibition was 51% in peripheral blood and 70% in peripheral tumor tissue. This trial did not lead to an expansion study and there is no current neoadjuvant olaparib (monotherapy) trials.

Unlike olaparib, talazoparib has been tested in neoadjuvant monotherapy trials in breast cancer. A feasibility study was commenced in 2017 to assess the activity of single-agent talazoparib over 24 weeks and recruited 20 patients with operable HER2 negative, *gBRCAm,* breast cancer (NCT02282345).[45] The outcome measure used in this study was residual cancer burden (RCB) with 53% achieving pCR. This proved that delivery and assessment of talazoparib monotherapy was feasible in this setting and a follow-on phase II expansion study was initiated (NEOTALA - NCT 03499353) enrolling 61 patients with stage II-III HER2 negative, *gBRCAm,* breast cancer. The study's primary endpoint was pCR evaluated by an independent central review. This study reported a pCR rate of 49.2% (80% CI 40.97–57.39).[46]

Further neoadjuvant PARPi monotherapy trials are ongoing and include a phase II non-randomized open-label trial (NCT03329937) which has recruited 21 patients with stage 2 to 3 HER2 negative, *gBRCAm,* breast cancer. Patients in this study receive niraparib monotherapy for 2 months before surgery. The primary outcome measure is tumor response seen on MRI before surgery and secondary outcome is pathologic response in the surgical specimen. The results of this study are awaited.

Neoadjuvant poly-(ADP ribose) polymerase inhibitor and chemotherapy combinations

PARPi plus cytotoxic chemotherapy combinations can be challenging to deliver due to issues with toxicity, most notably myelosuppression. However, the I-SPY2 group has reported the feasible combination of the clinical PARPi, veliparib, in combination with carboplatin (VC) and paclitaxel followed by AC in a trial with a Bayesian adaptive design and compared pathologic response outcomes between two groups—either receiving VC or not. The study met the prespecified requirements for graduation to a phase III trial. I-SPY2 reported pCR in 51% of patients who received VC versus 26% without VC in the TNBC subgroup.[47] Of note, although veliparib is an effective inhibitor of PARP1 catalytic activity, its ability to trap PARP1 and elicit a BRCA1/2 synthetic lethal effect in vitro is limited when compared with other clinical PARPi,[31,32] which could explain why such a PARPi/chemotherapy combination is achievable with veliparib.

A further phase III trial, BrighTNess, reported the efficacy of adding veliparib-carboplatin combination to paclitaxel (segment 1) followed by the SOC NACT regimen AC (segment 2) in patients with stage 2 to 3 TNBC. This trial allowed the contribution of veliparib to be dissected away from that of carboplatin by comparing three treatment groups: (i) VC + paclitaxel (VCP); (ii) carboplatin + paclitaxel (CP); and (iii) paclitaxel (P) alone.[48] Randomization to segment 1 was stratified by *gBRCA1/2m* status, nodal stage, and planned schedule for AC administration. A higher pCR rate was seen in the VCP group than in the P group (53% vs. 31%, $P<.0001$). However, the CP group achieved a pCR rate of 58%, indicating that the increase in pCR achieved by VCP was most likely due to C. A subsequent secondary analysis report at a more mature follow-up focused on an event-free survival (EFS) endpoint and confirmed that the addition of veliparib to carboplatin and paclitaxel did not enhance efficacy over carboplatin and paclitaxel alone.[49] There was a strong relationship between pCR and improved EFS that was unaffected by *gBRCA1/2m* status. As such, it appears the addition of carboplatin improves pCR and EFS in stage II/III TNBC regardless of *gBRCA1/2m* status

and should be offered to high-risk TNBC with *gBRCAm*. We speculate that the effect of platinum across an unselected group of early TNBCs regardless of *gBRCA1/2m* status in both BrighTNess and GeparSixto[40,48] may reflect the frequency with which HR is impaired by other genetic and epigenetic mechanisms in early TNBC.[50] Of note the BrighTNess trial has clearly indicated there is no benefit to addition of low trapping potency PARPi such as veliparib, despite the feasibility of combining this PARPi with carboplatin and paclitaxel.

The PARTNER trial is a phase II/III open-label RCT, testing the potent PARP trapping PARPi olaparib in combination with carboplatin and paclitaxel in the neoadjuvant setting in TNBC including *gBRCA1/2m* carriers. PARTNER has recently presented the preliminary safety data from the first 2 stages of this trial, describing a manageable toxicity profile for a regimen of low dose of olaparib with brief and intermittent administration of carboplatin.[51] The most common adverse event with this regimen was hematological; CTCAE grade 3 (G3) neutropenia was noted in 19% of patients, anemia in 15% and thrombocytopenia in 5%. The trial has now completed accrual of its third stage, which evaluates efficacy through measurement of pCR rate but is yet to report its findings.

GeparOLA, a phase II RCT, reported the safety and efficacy of a reduced but continuous dose olaparib used in combination with 12 weeks of weekly paclitaxel compared with paclitaxel plus carboplatin (AUC2) ahead of four cycles of standard or dose-dense epirubicin-cyclophosphamide (EC) chemotherapy as neo-adjuvant chemotherapy before definitive surgery. This trial included patients with HER2 negative stage 2 to 3 breast cancer with either *gBRCAm* or a mutational signature of homologous recombination deficiency (HRD). The paclitaxel and olaparib group achieved modestly higher pCR rates when compared with paclitaxel and carboplatin group (55.1% vs. 48.6%), although this did not reach the prespecified pCR rate of greater than 55%.[52] This high level of pCR was achieved with significantly fewer serious adverse events (SAEs) (13% vs 51%) than with the standard paclitaxel and carboplatin regimen. Of note, the hormone receptor-positive population of patients with HR-deficient breast cancer achieved pCR of 52.6% with olaparib compared with 20% with carboplatin. This provides a strong rationale for continuing to investigate the combination of a potent PARP1 trapping inhibitor in place of carboplatin in larger phase three trials in HR deficient breast cancer.

Adjuvant poly-(ADP ribose) polymerase inhibitor monotherapy

The OlympiA study (NCT02032823) recruited patients with high-risk HER2 negative breast cancer, with germline pathogenic or likely pathogenic *BRCA* mutations; these were randomized to receive either 12 months of postoperative olaparib or placebo in a double-blind design. The prespecified interim analysis (at 2.5 years median follow-up) described significant improvements in invasive disease-free survival (IDFS), HR 0.58 (99.5%CI 0.41–0.82; $P<.001$) and distant disease-free survival (DDFS), HR 0.57 (99.5%CI 0.39–0.83, $P<.001$).[53] Since this, the second prespecified analysis of OS and updated invasive disease-free survival (IDFS) associated with a median follow-up of 3.5 years has been reported. This analysis indicated that olaparib significantly improved overall survival (OS) with an HR for invasive disease or death of 0.68 (95%CI 0.47–0.97; $P = .009$) and showed that the IDFS and DDFS benefits seen in the first interim analysis were maintained.[54] This is the first trial reporting the OS benefits of olaparib as monotherapy in the adjuvant setting and has led to an FDA approval as an adjuvant therapy after chemotherapy in patients with high recurrence risk, HER2 negative, *gBRCA1/2m* early breast cancer and changed treatment guidelines from ASCO,[55] ESMO,[56] NCCN[57], and the St Gallen Consensus panel.[58]

Adjuvant chemotherapy

Masuda *and colleagues* reported the efficacy of adjuvant capecitabine in TNBC patients with HER2 negative breast cancer who have residual disease after NACT (CREATE-X).[59] Patients were randomized to receive oral capecitabine in addition to standard post-surgical treatment, or no capecitabine (control). DFS in the TNBC subgroup was longer in the capecitabine group than in the control cohort (69.8% vs. 56.1%, HR 0.58 (95%CI 0.39–0.87)). HR for death at 5 years was 0.52 (95% CI 0.30–0.90). There were no subgroup analyses looking at a *BRCA* deficient cohort; however, this trial was the first of its kind, reporting the efficacy of a second adjuvant chemotherapy in the high-risk TNBC subpopulation with residual disease after NACT. GEICAM-CIBOMA was a phase III RCT with a different design which looked at the value of adding capecitabine following SOC NACT in patients with TNBC; analysis of this study indicated that additional benefit was limited to the non-B-L subgroup and was not significant overall.[60]

The safety of a capecitabine and olaparib combination is untested and so physicians face making a choice as to whether patients with *gBRCAm* and residual TNBC after NACT will benefit more from capecitabine chemotherapy over olaparib in the early setting. Data from advanced disease trials, discussed below (OlympiAD and EMBRACA), in which the SOC chemotherapy backbone was most commonly capecitabine, suggest that olaparib significantly outperforms capecitabine in the metastatic setting in *gBRCAm* breast cancer and that the performance of adjuvant capecitabine in B-L breast cancer in the second adjuvant setting appears very poor. As a result, we suggest olaparib would seem the more appropriate choice given the high frequency of B-L breast cancer in *gBRCAm* carriers.[61]

Systemic Treatment in Advanced Breast Cancer

Chemotherapy in advanced breast cancer

When used in the advanced setting, platinums have been shown to elicit profound responses when compared with taxanes. A small phase II study carried out in 2012 reported the efficacy of single-agent cisplatin chemotherapy in *gBRCA1m* metastatic breast cancer. Patients were treated with 6 cycles of cisplatin; the overall response rate (ORR) was 80% (18 of 20 patients); 45% (9 of 20 patients) achieved a complete clinical response with 35% (7 of 20 patients) experiencing a partial response.

In 2015 the CBCSG006 phase III RCT was published. This study compared cisplatin + gemcitabine versus paclitaxel + gemcitabine in metastatic TNBC and found that cisplatin + gemcitabine was superior to paclitaxel + gemcitabine (HR for PFS of 0.692, 95% CI 0.523–0.915, p superiority = 0.009). The study group concluded that platinum-based chemotherapy in conjunction with gemcitabine should be considered as first-line treatment of mTNBC.[43]

The phase III RCT, TNT, assessed the role of carboplatin versus SOC docetaxel in advanced TNBC. Patients with *gBRCAm* in the carboplatin-treated group had a significantly improved progression-free survival (PFS) in comparison to the SOC group (6.8 months vs 4.4 months; $P = .002$). In addition to convincing data generated in the *gBRCAm* group, patients were also prespecified to be analyzed as putative 'BRCAness' subgroups in which their *BRCA1*-methylation status and their HRD genomic (HRD) score were taken into account. In contrast to the *gBRCAm* cohort of patients, these groups seemed to derive no selective benefit from platinum agents over docetaxel; one hypothesis is that in the BRCAness but not *gBRCAm* patients, the loss of epigenetically driven BRCAness during disease evolution from primary to the metastatic setting might reverse an HR defect and prevent carboplatin sensitivity.[23] Following the publication of the TNT trial, guidelines have included recommending

the use of platinum chemotherapy for patients with *gBRCAm* in early and advanced breast cancer.[56,62]

Poly-(ADP ribose) polymerase inhibitor monotherapy in advanced breast cancer

In 2009 Fong *and colleagues* reported the safety and efficacy of olaparib monotherapy in the phase I study of olaparib in advanced previous treatment-refractory solid tumors. The study enrolled 60 patients overall, including 23 with *gBRCAm,* three of whom had breast cancer. No objective responses were seen in the non-*BRCAm* group, whereas 63% of *gBRCAm* patients derived clinical benefit from olaparib.[63] Following publication of this data, two phase II trials were carried out showing proof of concept and tolerability for the efficacy of single agent olaparib in advanced stage breast and ovarian cancer.[64,65]

FDA approval was awarded for olaparib monotherapy in the advanced breast cancer setting following the publication of the OlympiAD trial in 2017. This phase III open-label RCT compared olaparib monotherapy using 300 mg in a tablet formulation twice daily with SOC chemotherapy in patients with HER2 negative stage 4 breast cancer with *gBRCAm*. The group reported a significantly prolonged PFS in the olaparib group versus SOC chemotherapy (7.0 vs 4.2 months), HR for disease progression or death was 0.58 (95%CI 0.43–0.80; $P<.0001$).[66] In 2019 updated OS data were published, which revealed a median OS of 19.3 months in the olaparib arm versus 17.1 months in the SOC chemotherapy arm, the difference in OS was not statistically significant overall but with suggestion of an OS benefit in a pre-the specified subgroup who had received no prior chemotherapy for advanced disease.[67] As olaparib has proven effective in *gBRCAm* breast cancer, work has been carried out to determine its use in patients with somatic tumor mutations in *BRCA1* or *BRCA2* (*sBRCA1/2m*) or in those with germline mutations in other HR genes (eg, *gPALB2m*). The TBCRC-048 study reported the use of olaparib in 54 patients with either *sBRCA1/2m*, or germline pathogenic mutations in other HR-associated genes. An ORR of 50% was seen in those with *sBRCA1/2m* ($n = 16$), and ORR of 82% in the *gPALB2m* group. Interestingly, no responses to olaparib were seen in those with *ATM* ($n = 8$), *CHEK2* ($n = 8$) mutations or in those with both *ATM* and *CHEK2* ($n = 2$) mutations.[68]

Talazoparib has also been compared with SOC chemotherapy in a phase III RCT (EMBRACA). This study included patients with stage 4 HER2 negative breast cancer with *gBRCAm*. Patients must have received no more than 3 lines of prior chemotherapy (which must have included an anthracycline or taxane). The group reported a median PFS of 8.6 months versus 5.6 months in the talazoparib group versus SOC chemotherapy. HR for death or disease progression was 0.54 (95%CI 0.41–0.71; $P<.001$).[69] Talazoparib has since been approved by regulatory authorities for use in advanced breast cancer patients with *gBRCAm*. Studies into the efficacy of niraparib in the advanced setting have been less successful. BRAVO, a phase 3 RCT, reported the efficacy of niraparib (a PARPi with similar trapping potency to olaparib) monotherapy in *gBRCAm* HER2 negative stage 4 breast cancer. Unfortunately, there was a discrepancy between the central and local reviewers in determining the progression of the disease, and as such the primary endpoint of PFS was noncomparable between study arms and the investigators terminated recruitment early.[70]

Poly-(ADP ribose) polymerase inhibitor and chemotherapy combinations in advanced breast cancer

Delivering PARPi in combination with chemotherapy, especially those that induce DNA damage, has proven challenging because of the additive or synergistic effects of treatment on rapidly proliferating bone marrow cells. When olaparib was combined

with paclitaxel in a phase I dose escalation trial, the investigators reported dose-limiting hematological toxicity when olaparib was delivered at 200 mg twice daily for a 28-day continuous treatment in combination with paclitaxel delivered once weekly (G3 neutropenia was reported in 44%). However, when G1 or above neutropenia was recorded after the first cycle of treatment, patients were supplemented with granulocyte colony-stimulating factor (GCSF) and G3 neutropenia reduced to 20%.[71]

The phase II RCT, BROCADE, assessed the use of veliparib in addition to carboplatin and paclitaxel (VCP), or temozolomide (VT) and compared this to placebo + carboplatin + paclitaxel (PCP) in patients with stage 4 breast cancer of all subtypes, with *gBRCAm*. In the VCP versus PCP analysis, the median PFS was 14.1 months versus 12.3 months, HR 0.78 (95%CI 0.536–1.1.62), $P = .227$. ORR in the VCP group was higher than PCP; 77.8% versus 61.3%, $P = .027$. In comparison, the median PFS was 7.4 months in the VT group (HR 1.85, 95%CI 1.278–2.702, $P = .001$) and ORR of 28.6% ($P<.001$).[72] The clear inferiority of treatment with temozolomide and a taxane led to its omission from the phase III RCT, BROCADE3, which recruited patients with stage 4 *gBRCAm*, HER2 negative, breast cancer to receive VCP or PCP. The investigators reported a small improvement in PFS in the VCP group in comparison to PCP (14.5 months vs. 12.6 months, $P = .0016$, OS is awaited).[73] Closer examination of the survival curves indicates that separation only occurs after platinum therapy was stopped and veliparib was continued, suggesting that it is the ability to continue PARPi as opposed to platinum chemotherapy that leads to delay of progression. It is likely that this combination is tolerated better than other PARPi because of the weak PARP1 trapping effects of veliparib. A selection of published phase III RCTs summarizing systemic treatment of advanced breast cancer are found in **Table 2**.

Poly-(ADP Ribose) Polymerase Inhibitor and DNA Damage Response Inhibitors

Preclinical studies suggest that kinase inhibitors that cause replication stress and impair cell cycle checkpoints for example, ATR and WEE1 inhibitors, enhance antitumor cell efficacy in models with HR deficiency.[74–77] On the basis of this work, the VIOLETTE study (NCT03330847), a multicenter open-label phase II RCT was initiated. The study assessed the safety and efficacy of ceralasertib (ATRi) plus olaparib, or adavosertib (WEE1i) plus olaparib versus olaparib monotherapy in mTNBC in three distinct cohorts defined by validated or proposed biomarkers of HR deficiency in tumor sequencing. The trial recently reported no statistically significant difference observed in PFS for ceralasertib plus olaparib versus olaparib monotherapy as 2nd/3rd line therapy for mTNBC in any cohort. There was also no statistically significant difference observed in ORR for ceralasertib + olaparib versus olaparib monotherapy in the *BRCAm* group or the non-*BRCAm* (but other HR gene-deficient) group. A signal of improved response was seen in the non-HR deficient TNBC group and is currently being explored in deeper translational analysis.[78]

Phosphoinositide 3-kinase inhibitors (PIK3i) are currently being assessed as anticancer treatments in a variety of cancers. Recent data has shown that mutations in *RAS* and *PIK3CA* oncogenes could induce an HR defect and suggested that the inhibition of such proteins may induce a drug-induced form of BRCAness.[79] Several trials are ongoing investigating the combination of PI3K/AKTi in combination with PARPi (NCT04729387, nCT02208375, NCT04586335, NCT03586661) in solid tumors with *BRCAm* and could inform future treatment in *gBRCAm* breast cancer.

Poly-(ADP Ribose) Polymerase Inhibitor and Immune Checkpoint Inhibitors

Recent work has highlighted the contribution of the immune system to the efficacy of PARPi.[80–82] There are several trials underway examining the safety and efficacy of

Table 2
Clinical trials published in advanced breast cancer

Trial Name	Phase	Treatment	Setting	Endpoint	Key Results	Authors (Year)
TNT	III	Carboplatin vs docetaxel	Advanced	ORR and PFS	*gBRCAm* ORR 68% vs 33%; PFS 6.4 vs 4.4 mo	Tutt et al.[23]
OlympiAD	III	Olaparib vs SOC	Advanced	PFS and OS	PFS 7.0 vs 4.2 mo; OS 19.3 vs 17.1 mo	Robson et al.[67]
EMBRACA	III	Talazoparib vs SOC	Advanced	PFS	PFS 8.6 vs 5.6 mo	Litton et al.[69]
BROCADE 3	III	Veliparib + carboplatin + paclitaxel vs Placebo + carboplatin + paclitaxel	Advanced	PFS	PFS 14.5 vs 12.6 mo	Dieras et al.[73]

Abbreviations: ORR, overall response rate; OS, overall survival; PFS, progression free survival; SOC, standard of care.

PARPi in conjunction with immune checkpoint inhibitors. The MEDIOLA trial reported on the 12-week disease control rate (DCR) observed by adding durvalumab to olaparib in patients with advanced solid tumors. MEDIOLA reported a 12-week DCR of 80% in those receiving the combination, which surpassed the prespecified target of 75%.[83] TOPACIO/KEYNOTE-162, a phase I/II trial, reported the use of pembrolizumab in addition to niraparib in patients with advanced TNBC or ovarian cancer. This trial reported an ORR of 18%, with a 12-week DCR of 65%.[84] There are many further ongoing trials reporting the efficacy of adding immune checkpoint inhibitors to PARPi.

Poly-(ADP Ribose) Polymerase Inhibitor and Platinum Resistance

Despite PARPi and platinum agents both inducing objective response rates of more than 60% in HR deficient breast cancers, the lack of meaningful response in approximately one-third of patients and median duration of response in those who do respond being approximately 6 months indicates that *de novo* and acquired resistance is a significant problem.[67,69] Understanding the biology of the underlying resistance mechanisms is vital and will help aid treatment decisions. Considerable preclinical work has identified mechanisms of resistance to PARPi and/or platinum salts, including mutations in PARP1 that prevent PARP1 trapping,[85] loss of *TP53BP1* or the shieldin complex which normally prevents DSB resection,[86–89] upregulation of drug efflux transporter genes *Abcb1a* and *Abcb1b* which encode for efflux pumps MDR1/P-gp and *Abcg2,*[90] loss of the PAR glycolase PARG[91] loss of DNA end protection and restoration of replication fork stability through loss of MRE11 and MUS81[92,93] or secondary, reversion, mutations in HR genes that restores their function.[94,95] Of these, reversion mutations are perhaps the most clinically validated mechanism of PARPi resistance; these reversions also cause cross-resistance to platinum-salts as they restore HR. For example, under the selective treatment pressure of PARPi and platinums, reversion mutations in *BRCA1*, *BRCA2, PALB2* or the *RAD51* paralogs *RAD51 C* and *RAD51D* have been identified.[96–99] These reversion mutations are secondary mutations (ie, mutations in addition to the pathogenic mutation) which restore the open reading frame of the gene, thereby restoring HR, rendering PARPi or platinum salts ineffective. More recently, detection of reversion mutations in plasma-derived cell-free tumor DNA (ctDNA) from patients with clinical PARPi resistance has been shown[100–102] providing a useful non-invasive method of tracking resistance to treatment in the clinic.

Future Directions

Biomarker development in hereditary breast cancer

Although there is a growing body of preclinical work that identifies possible mechanisms of PARPi resistance, whether all of these operate in the clinical disease, and at what frequency, remains unknown. Given this, molecular profiling (DNA/RNA sequencing, proteomics, and methylation) of biopsies from individuals who develop resistance to drugs used in the treatment of hereditary breast cancer is required to clarify this area. This could lead to the development of clinical biomarkers that help predict response to PARPi. Current biomarkers used to guide the use of PARPi in breast cancer include the identification of pathogenic *gBRCA1/2m*, whereas in other cancers, the presence of mutations in other HR genes or the presence of a genome-wide mutational scar reflective of a past or extant HR defect are used. Whether such biomarkers could be used to direct the use of PARPi in hereditary breast cancer remains to be seen. Similarly, functional characterization of tumoral HR function through the use of immunohistochemical analysis of RAD51 and the development of

biomarkers that predict dose-limiting toxicity (DLT) could lead to improvements in the way PARPi and platinum salts are used.

Germline PALB2 mutation carriers

Although there are no specifically approved targeted treatment options for *gPALB2m* breast cancer, given the integral role of PALB2 in HR, recent studies have suggested treatment with PARPi may be beneficial.[68] For example, a phase 3 RCT (PROFOUND) evaluating the efficacy of the PARPi olaparib in men with castration-resistant prostate cancer established that patients with mutations in HR genes other than *BRCA1/2*, including *PALB2*, receive some benefit from olaparib treatment.[103] The ACMG has recently recommended consideration of *gPALB2m* as equivalent to *gBRCA1/2m* for therapy decisions including systemic therapy decisions.[104] Trials in early breast cancers with pathologic response or biomarker efficacy endpoints that can prove equivalence of concept of treatment effects of PARPi between *PALB2* and *BRCA1/2* mutation carriers, may allow the avoidance of the need to repeat large phase III studies in specifically *PALB2* mutation carriers.

Novel combination therapies

As we have discussed earlier, DLT is a significant issue limiting the delivery of potent PARPi combination therapies. Recent data from a Phase I study in patients with *BRCA1/2, PALB2* or *RAD51 C*/D mutations presented at AACR in April 2022 (PETRA study, NCT04644068) suggest a new highly selective PARP1 inhibitor, AZD5305, can elicit efficacy with low rates of grade 3 treatment-emergent adverse events, SAEs or discontinuations.[105] This enhanced tolerability might be because the myelosuppression associated with other PARPi is mediated through effects on PARP2 and other PARP family members, opening up the possibility that this optimized PARPi could provide a clinical agent that is not only an effective PARP1 trapping agent, but which also delivers tolerable regimens when combined with DNA damaging chemotherapy.

The development of drug combinations to target PARPi resistance including delivery of PARPi with other small molecule inhibitors of oncogenic drivers and survival or DDR pathways[79,106–109] and also in combination with immunomodulatory agents and radiotherapy[83,84,110] will remain an very appropriate area for future clinical studies.

SUMMARY

Treatment options for patients with hereditary breast cancers have expanded significantly in the past 20 years. These include a new FDA-licensed approach to adjuvant therapy, olaparib, that improves OS in those with *gBRCA1/2m*. However, resistance to platinum-based chemotherapy and PARPi in this cohort of patients is an issue that requires considerable focus. We have discussed standard approaches to treatment, but have touched on the expanse of pre-clinical and early clinical work being undertaken which will likely shape future treatments in this field of medicine.

CLINICS CARE POINTS

- Patients with breast cancer, whether hormone receptor positive or negative should be considered for referral for genetic counselling and testing using agreed international criteria eg. NCCN as it may affect their systemic treatment recommendations.
- Patients with hereditary breast cancer due to germline *BRCA1* and *BRCA2* mutations should be reassured that they do not have worse prognosis than those with similar forms of breast

cancer without such mutations and have better prognosis when treated with standard adjuvant chemotherapy regimens.

- In germline *BRCA1* or *BRCA2* mutation carriers both platinum chemotherapy and the PARP inhibitors olaparib and talazoparib are associated with high response and showed improved progression free survival compared to standard of care advanced disease chemotherapy regimens, but have not been directly compared with one another.
- PALB2 is a protein with similar functions in homologous recombination DNA repair to BRCA2 and *PALB2* mutation carriers with advanced breast cancer have similar response to PARP inhibitors to *BRCA1* and *BRCA2* mutation carriers4. International guidelines indicate that patients with germline *BRCA1* or *BRCA2* mutations high risk early breast cancer and should be offered adjuvant olaparib for 12 months following completion (neo)adjuvant chemotherapy and local therapy (including surgery and radiotherapy).

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