PARP inhibitors and breast cancer – highlights and hang-ups

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Abstract

Introduction: PARP inhibitors (PARPi) have been developed clinically as a treatment for cancers with defects in the DNA repair processes controlled by the *BRCA1* and *BRCA2* tumour suppressor genes. Although the path from initial pre-clinical proof of concept through to clinical approval for PARPi in breast cancer has not been straightforward, recent clinical trial data suggests that some of the initial enthusiasm for developing these drugs in this disease is starting to return. **Areas covered:** Here, we review published pre-clinical and clinical data pertaining to the use of PARPi in breast cancer, including: the mechanism of action of PARPi; predictive biomarkers associated with drug sensitivity; clinical trials of PARP inhibitors in breast cancer, and mechanisms of drug resistance and drug combination strategies. **Expert commentary:** The recent publication of promising phase III clinical trial data describing a progression free survival benefit in breast cancer patients with *BRCA* gene mutations treated with a PARP inhibitor suggests that the comparative drought of clinical data in this setting is coming to an end. The task ahead is now to optimise PARPi therapy, in terms of identifying the ideal drug combinations to use with these agents as well as establishing the optimal stage and scheduling that should be used to achieve maximum benefit and manage the emergence of resistance.

1. Introduction

1.1 PARP inhibitors: mechanism of action and predictive biomarkers

The principal target of most clinical PARP inhibitors (PARPi) is PARP1, an abundant and largely nuclear protein that detects and signals DNA damage ([1] and references therein). PARP1 binds in milliseconds to sites of single and double stranded DNA breaks, whereupon it synthesises long chains of poly(ADP-ribose) (PAR) using β-NAD^+ as a substrate [2]. The long, negatively charged PAR polymers that form recruit effectors of DNA repair, such as XRCC1, and ultimately promote dissociation of PARP1 from the damage site so that repair can occur [2, 3]. PARP1 activity is crucial for the repair of single stranded DNA breaks (SSBs) and DNA lesions caused by alkylating agents such as dimethyl sulphate [4], observations that in part provided the rationale for the discovery of drug-like PARP1 inhibitors that could potentially be used to enhance chemo- or radiotherapy responses in cancer [5]. PARP inhibitors also alter the behaviour of vascular cells and inflammatory processes (reviewed in [6]), both of which could conceivably modulate the anti-tumor efficacy of these agents, as well as potentially providing the rationale for using PARP inhibitors to target non-cancer related diseases [7].

The later discovery that tumour cells with mutations in either the *BRCA1* or *BRCA2* breast and ovarian cancer susceptibility genes are extremely sensitive to small molecule PARPi provided the rationale for developing these agents for treating for *BRCA* gene mutant (*BRCA1 or BRCA2*; *BRCAm*) breast or ovarian cancers [8-10]. When cells enter S phase with a high load of unresolved DNA damage caused by PARPi exposure, this leads to replication fork stalling and collapse, generating DNA lesions that are normally resolved by homologous recombination (HR). HR, a DNA repair process that is mediated by the RAD51 DNA recombinase, relies upon BRCA1 and BRCA2. Therefore cells without functional BRCA1, BRCA2 or indeed other proteins

that mediate HR, cannot effectively process the DNA damage caused by PARP1 inhibitors, making them exquisitely sensitive to these agents.

Auto-PARylated PARP1 is unable to bind damaged DNA [11] whereas unmodified PARP1 can bind to sites of damage under conditions where PAR synthesis is inhibited [1, 4, 12], observations consistent with the hypothesis that auto-PARylation of PARP1 is required for PARP1 to dissociate from DNA, and that PARP inhibitors could result in stabilisation of PARP binding to damaged DNA. More recently this has become known as the trapping hypothesis, and the differing cytotoxic potencies of different clinical PARP inhibitors have been shown to correlate with their differing ability to “trap” PARP1 on damaged DNA [13, 14]. Taken together, this has been interpreted as reflecting inhibited PARP1 being stably bound to sites of DNA damage, unable to synthesise the PAR chains that would otherwise allow PARP1 to recruit repair factors and eventually dissociate from the damage site. This "trapped" PARP1 is likely to severely impede replication fork progression and preclude repair of the DNA damage by any other pathway, and therefore represents a potentially cytotoxic DNA lesion. In support of this hypothesis, much of the cytotoxicity of clinical PARP inhibitors observed in BRCA1/2 wild type cells can be prevented by loss of *PARP1*, suggesting that PARP1 itself is required for PARPi-mediated cytotoxicity [13, 15].

Although the original rationale for using PARP inhibitors to treat breast cancer was based on the sensitivity of *BRCA1* and *BRCA2* mutant tumour cells to these drugs, the utility of PARP inhibitors could extend beyond *BRCA1/2* mutant patients. One year after the identification of BRCA/PARP synthetic lethality, McCabe *et al* demonstrated that defects in other DNA repair genes commonly found in human cancers, including those involved in DSB detection and repair (e.g. *ATM, RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, or FANCC*), also conferred PARPi sensitivity upon cells [16], observations later confirmed and extended in genome-scale genetic screens where defects in additional DNA repair related genes mutated in ovarian cancers, such as *CDK12,* were found to cause PARPi sensitivity [17]. These observations were consistent with the

"BRCAness" hypothesis, which postulates that a subset of cancers in patients without germline *BRCA1* or *BRCA2* mutations display histopathological, molecular and clinical similarities, including drug sensitivity phenotypes, with germline BRCAm cancers (recently reviewed in [18]). This BRCAness concept seems to be relevant in terms of clinical PARP inhibitor sensitivity; in high-grade serous ovarian cancers (HGSOvCa), clinical responses to PARP inhibitor have been seen in patients with loss of function *RAD51C* or *RAD51D* mutations [19, 20]. Furthermore, up to a fifth of advanced prostate cancers have germline or somatic mutations in DNA repair genes that could potentially cause PARP inhibitor sensitivity [21] and clinical responses to PARPi have been observed in patients with metastatic, castration-resistant, prostate cancers with mutations in BRCAness-associated genes including *FANCA*, *PALB2* and *ATM* [22].

In addition to using germline or somatic gene mutations to explain clinical PARPi responses, some clinical trials have started to assess the potential for using tumour-specific patterns of mutation to predict PARPi responses. Tumours in individuals with *BRCA1* or *BRCA2* germline mutations tend to exhibit mutations and chromosomal aberrations (e.g. large scale loss of heterozygosity (LOH) effects) that are somewhat reflective of the use of error-prone forms of repair in the absence of functional homologous recombination; similar mutational patterns also exist in tumours, including breast tumours, with somatic *BRCA1* or *BRCA2* mutations and in tumours without detectable *BRCA* gene dysfunction [23-25]. These observations suggest that such mutational signatures could be used to direct the use of therapies, such as PARP inhibitors, that target homologous recombination defects. The recently described ARIEL3 phase III trial of rucaparib in advanced ovarian cancer [26] included a prospective assessment of LOH as measured by a tumour DNA sequencing assay (Foundation Medicine T5 assay [27]) and found greater benefit for *BRCA* wild type patients with high LOH compared to those with low LOH, although both groups showed improved progression-free survival (PFS) compared to placebo. The RUBY trial phase II trial of rucaparib in metastatic breast cancer (NCT02505048) will also assess a genomic signature to predict the response to a PARP inhibitor.

1.2 Regulatory approvals for PARP inhibitors in gynaecological cancers

Several PARP inhibitors have now been approved for use in patients with gynaecological cancers. In general, these approvals allow PARP inhibitors to be used in patients after the use of classical chemotherapies, including platinum-based treatments (Table 1). The first PARP inhibitor to be approved for use was olaparib (Lynparza, KuDOS/AstraZeneca); this was first approved for use by the European Medicines Agency (EMA) in 2014 as a treatment for platinum-sensitive, relapsed, *BRCA1* or *BRCA2*-mutated (germline and/or somatic) high grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancers (Table 1). This approval was swiftly followed by a similar Federal Drug Agency (FDA) approval that allowed olaparib to be used in patients with deleterious germline *BRCA*-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy. These decisions heralded the first approval of a synthetic lethal treatment for cancer, the first approval of a cancer drug for an inherited cancer predisposition syndrome, as well as the first approval of a cancer treatment with a companion predictive genetic biomarker test, namely the assessment of *BRCA1/2* gene DNA sequence [10]. On the basis of SOLO-2 trial results [28, 29], the approved use of olaparib was recently expanded to allow patients with recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer, who are in a complete or partial response to platinum-based chemotherapy to receive olaparib as a maintenance treatment after platinum-based therapy – regardless of whether they have a confirmed *BRCA* gene mutation. Rucaparib (RUBRACA, Clovis Oncology Inc.) was granted accelerated approval for the treatment of advanced ovarian cancers with germline or somatic *BRCA* mutations in 2016 based on ARIEL2 and other phase II data [20, 30, 31]. Niraparib (Zejula, Tesaro Inc.) was also approved for use this year, based on results from the NOVA trial [28] as a maintenance therapy for recurrent epithelial ovarian, fallopian tube or peritoneal cancer.

Veliparib (ABT-888, AbbVie Inc.) has yet to be approved for the treatment of cancer, but has been given orphan drug designation for the treatment of advanced squamous non-small-cell lung cancer (NSCLC). Veliparib is noteworthy for its relative lack of PARP1 "trapping" properties compared to other clinical PARP inhibitors, despite being an effective catalytic inhibitor of the protein [13, 14]. Another PARP inhibitor that is still in early stage trials, talazoparib [32], displays opposite characteristics: high trapping and cytotoxicity relative to its effect on PARP1 catalysis [14]. How these properties affect clinical efficacy and the deleterious side-effect profile of this class of drugs, remains to be determined. Finally, some trials carried out prior to 2011 used iniparib, a drug that was designed as a PARP inhibitor but has been subsequently shown to have limited PARP1 inhibitory activity [33].

Recently, the PARP inhibitor olaparib was approved by the FDA for use in breast cancer. This application is based largely on the results from the OlympiAD phase III clinical trial, discussed below. This review will focus on recent clinical and preclinical studies of PARP inhibitors in breast cancer that have led to this approval. Earlier PARP inhibitor clinical trials have been reviewed extensively, particularly in the case of ovarian cancer where PARPi clinical development is most advanced [34].

Table 1. PARP inhibitors in clinical use and development

Drug	Other names	Company	First Approval	FDA Approved indication(s)	PARP1 IC_{50} (nM)	Trapping potency
Olaparib	Lynparza® AZD2281 KU0059436	AstraZeneca	2014	Maintenance treatment of recurrent platinum sensitive epithelial, ovarian, fallopian tube, peritoneal cancer; Advanced g*BRCAm* ovarian cancer after three or more lines of chemotherapy; Metastatic g*BRCAm*, HER2-negative breast cancer after chemotherapy or endocrine therapy (as appropriate for hormone receptor status).	5	+
Rucaparib	RUBRACA® AG-014699 PF-01367338	Clovis Oncology Inc.	2016	Advanced g*BRCAm* ovarian cancer after two or more lines of chemotherapy	1.4	+
Niraparib	ZEJULA™ MK-4827	Tesaro Inc.	2017	Maintenance treatment of recurrent platinum-sensitive epithelial, ovarian, fallopian tube, peritoneal cancer.	3.8	+
Veliparib	ABT-888	Abbvie	2016	Orphan designation for NSCLC	5.2	–
Talazoparib	BMN-673	Pfizer	–	–	0.57	++

2. Clinical trials of PARPi in breast cancer

2.1 Initial Trials – highlights and hang ups

Soon after the identification of BRCA/PARP synthetic lethal effect in pre-clinical models [8, 9], phase I and II clinical trials confirmed that this effect also had considerable clinical potential. A phase I trial of olaparib, identified a number of significant and sustained anti-tumour responses in heavily pre-treated patients with *BRCA* gene mutant (BRCAm) advanced breast cancer; these clinical responses were also achieved with a less severe and more manageable side effect profile, when compared to classical chemotherapy. [35]. For example, of three *BRCA2* mutant breast cancer patients included in this trial, one exhibited a complete radiological response, while a second experienced prolonged disease stabilisation when treated with olaparib. A subsequent phase II trial (ICEBERG) using 400 mg olaparib twice daily in patients with *BRCAm* advanced breast cancer*,* elicited an impressive 41% objective response rate (ORR, [36]), which was comparable to a response rate of 33 % in a similarly treated *BRCAm* advanced ovarian cancer phase II clinical trial [37]. In the phase II breast cancer trial, responses to olaparib were seen in patients with either estrogen receptor (ER) positive or negative tumours, suggesting that estrogen receptor status was less important in determining PARP inhibitor synthetic lethality in breast cancer than *BRCA* gene status. Within the same clinical trial, another cohort of patients received 100 mg olaparib twice daily and had a slightly lower ORR (22%), suggesting that the higher olaparib dose be used for subsequent clinical trials.

Despite this promising data, a subsequent phase II trial of olaparib that included both breast and ovarian cancer patients observed no objective responses amongst eight *BRCAm* breast cancer patients, despite shrinkage of the target lesion in six cases and encouraging results in the ovarian cancer patients - 41% of *BRCAm* ovarian cancer patients responded, and the response rate was also high in non-BRCA mutants [38]. A more recent phase II study investigating different methods (intravenous compared to oral) and

scheduling of rucaparib dosing also included both breast and ovarian patients, and again observed that objective responses were limited to the ovarian cancer patients, with stable disease being the best outcome in breast cancer patients (9/23 patients, [39]). A larger phase II trial, which included 62 germline *BRCAm* (*gBRCAm*) breast cancer patients, did report responses but at a lower rate than the ICEBERG trial of 12.9% when patients received 400 mg twice daily olaparib (Study 42, [40]). Taken together, these phase II results for PARP inhibitors in breast cancer were somewhat disappointing when viewed in the context of the higher response rate in ovarian cancer patients.

2.2 Phase III single agent studies: OlympiAD

Results from the OlympiAD study, the first phase III trial of a PARP inhibitor in advanced breast cancer patients with *BRCAm*, were recently reported [41]. This randomised, open-label, trial in 302 *BRCAm* metastatic breast cancer patients compared olaparib monotherapy to physician's choice of conventional standard of care chemotherapy (capecitabine, eribulin, or vinorelbine). Eligibility criteria for this trial included a germline *BRCA* gene mutation and the absence of *ERBB2* oncogene amplification/overexpression (HER2-negativity). *BRCA* gene mutation status was assessed using the Myriad BRACAnalysis test. Twenty-nine per cent of patients had previously received platinum chemotherapy - this was permitted by the criteria provided that there was no evidence of progression on platinum (in the metastatic case) or there had been at least 12 months since the last dose in the neoadjuvant or adjuvant setting. The primary endpoint of the trial was to assess progression-free survival (PFS).

Median PFS was significantly longer in the olaparib-treated cohort of patients, at 7.0 months, compared to 4.2 months in chemotherapy-treated patients (Hazard Ratio (HR) = 0.58). The response rate was also higher in the olaparib-treated cohort (59.9% vs 28.8%). The OlympiAD study also provided direct evidence of the favourable side effect profile of olaparib, when compared to chemotherapy in this patient population: the olaparib-treated cohort had a lower incidence of severe adverse events (Grade 3+ 36.6%

olaparib compared to 50.5% for chemotherapy-treated patients) and a lower frequency of treatment discontinuation related to toxicity (4.9% compared to 7.7%).

2.3 Overall survival and potential confounder effects

The OlympiAD trial did not show an increase in overall survival (OS), although it should be noted that this was not a primary endpoint and this trial was not designed to detect such a difference. Further long-term studies will be required to show whether PARPi treatment can prolong OS in breast cancer or whether the lack of OS improvement seen in OlympiAD could be due to additional factors not directly related to the overall effectiveness of a PARP inhibitor. For example, the OS of patients in a study such as OlympiAD is of course influenced by the treatments patients receive after completing the trial treatment regimen. A higher proportion of patients who received chemotherapy in the OlympiAD study received a platinum salt or a PARPi after leaving the OlympiAD study, compared to those who received olaparib as part of OlympiAD; it seems reasonable to think such a difference could influence OS estimates in open label trial designs such as OlympiAD. Notably the OlympiAD trial did include, although not as a primary endpoint, an analysis of investigator-assessed time to second progression or death (PFS2) which suggested that the olaparib treated group did have a better outcome after patients had completed the study, even though this did not ultimately result in an overall survival advantage (Median PFS2 13.2 months compared to 9.3, HR = 0.57, P = 0.0033). Even in a double blind ovarian cancer trial, the signal for OS benefit - assessed as a secondary outcome - was not statistically significant [42]. This may also be confounded by a large number of *BRCA* mutant patients in the placebo group later receiving PARPi therapy outside the scope of the trial after unblinding on progression [43].

2.4 EMBRACA

Results of the EMBRACA phase III trial of talazoparib in metastatic breast cancer were presented at the 2017 San Antonio Breast Cancer Symposium [44]. Trials for talazoparib have progressed at a faster pace than for olaparib. An ORR of 50% was observed in gBRCAm breast cancer patients in a phase

I study [45], followed closely by the ABRAZO phase II study, which reported an ORR of 21% and 37% in gBRCAm, platinum pre-treated and platinum-free breast cancer patients respectively [46].

The design of the EMBRACA study had many design similarities to the OlympiAD trial and was larger (431 patients compared to 302 for OlympiAD). Eligibility criteria included HER2-negative, metastatic or locally advanced cancer with a germline BRCA mutation. As with OlympiAD, hormone receptor positive patients were eligible provided they had received prior adjuvant therapy if appropriate, and slightly more such patients were recruited compared to OlympiAD (54% hormone receptor positive compared to 50%). Prior platinum treatment was also permitted (18% of patients, lower than the 28% of patients in OlympiAD). Patients in the two studies were of similar median age (OlympiAD: 44 years, EMBRACA: 46). EMBRACA included a slightly higher proportion of *BRCA2* mutant patients (55%) relative to *BRCA1* – in OlympiAD this was reversed (57% *BRCA1*). Patients were randomised in a 2:1 ratio between talazoparib and physician's choice chemotherapy. Choices in the chemotherapy arm were the same as in the OlympiAD study, with the addition of gemcitabine. However, as in OlympiAD, most patients in the chemotherapy group received capecitabine or eribulin. Serious (grade 3/4) adverse effects occurred at similar frequencies in both talazoparib and chemotherapy groups (25.5% compared to 25.4%).

As for OlympiAD, the primary endpoint of EMBRACA was PFS, and the results for this endpoint were similar. PFS was a median of 8.6 months in the talazoparib group compared to 5.6 months in the chemotherapy group (HR = 0.54, $p < 0.0001$). Unlike OlympiAD, EMBRACA was designed to detect differences in overall survival between groups. An interim analysis after 51% of projected events did not show a significant difference (HR 0.76, $p = 0.105$) although there was a trend towards improved survival with talazoparib at later times. It will be interesting to see if this continues in the final OS analysis. A planned subgroup analysis showed that the benefit of talazoparib was apparent for all subgroups, although as for OlympiAD the prior platinum-

treated subgroup had a wide confidence interval, suggesting that this group may contain some patients resistant to PARPi.

2.5 PARP inhibitors in breast *vs.* ovarian cancer – biological differences or clinical differences?

In discussing PARPi in breast cancer, it seems pertinent to discuss the differences in clinical responses and trial designs in *BRCA* gene mutant breast, as opposed to ovarian, cancer. Where similar clinical trials have been performed, for example in the ICEBERG1 (BRCAm advanced breast cancer, [36]) and ICEBERG2 phase 2 trials (BRCAm advanced ovarian cancer, [37]), response rates to olaparib were relatively comparable: 41% in breast cancer and 33% in ovarian cancer. However, beyond these two clinical trials, comparisons in the two diseases become somewhat difficult to interpret. For example, with the exception of rucaparib, where regulatory approval was granted based on phase II trial data [20, 31] subject to clinical benefit being shown in phase III ARIEL3 [26] and 4 trials, most ovarian cancer clinical trials using PARPi (and especially those that have supported FDA approvals) have compared PARPi treatment in patient populations with residual disease after chemotherapy, to a placebo treatment. These trials all showed improvements in PFS for PARPi treated *BRCAm* patients compared to those treated with a placebo, with Hazard Ratios ranging from 0.2-0.3 [26, 28, 29] in the respective *BRCAm* patient populations. In comparison, in advanced breast cancer, randomised clinical trials have always compared PARPi treated patients to a chemotherapy-treated comparator group - for example, capecitabine, eribulin, or vinorelbine treated patients in OlympiAD, where the HR was 0.58 in favour of olaparib. Insofar as clinical trials can be compared between different cancer types, a better comparison may be some of the earlier, non-maintenance therapy, ovarian cancer trials in which a measure of response rate is available. These trials include "study 12" [47],

which observed an ORR of 25-31% in *BRCA* mutant recurrent ovarian cancer; a trial of olaparib used in combination with the antiangiogenic cediranib (47% ORR in the olaparib-only arm of a population comprising about 50% *BRCA* mutant patients) [48] and the Gelmon study where an ORR of 41% was observed in the *BRCA* mutant ovarian cancer patients [38]. When examined in this light, the response rate seen in the breast cancer trial, OlympiAD (59.9%), compares favourably.

It is also important to point out the differences in how patients are selected for PARPi clinical trials in the two diseases. Although patient populations in ovarian *vs.* breast cancer clinical trials for PARPi share some unifying characteristics, such as a *BRCA1/2* mutation and advanced, metastatic, disease, the clinical histories of ovarian *vs.* breast cancer patients are very different. Most ovarian cancer patients who received PARPi in a clinical trial setting will have previously received at least two lines of platinum-based chemotherapy and are often selected for PARPi treatment by nature of their platinum salt sensitivity [49]. The importance of using prior platinum salt sensitivity as an enriching factor lies in the growing understanding that PARPi and platinum chemotherapies share some similarities in terms of mechanism of action. Both platinum salts and PARPi cause DNA lesions that stall replication forks and require BRCA1 and BRCA2 for their repair, interstrand, covalent, DNA crosslinks (ICLs) in the case of platinum salts and trapped PARP1 in the case of PARPi. Accordingly, platinum salts are also selectively cytotoxic to cells with homologous recombination defects, as HR is required as part of the pathway to restart replication forks during bypass of ICLs [50, 51]. Moreover, restoration of *BRCA1* or *BRCA2* gene function, by reason of reversion mutations in either of these two genes, causes clinical resistance to both PARPi and platinum salts [52-55]. Since the clinical trials that have led to PARPi approvals have all been carried out in patients with platinum sensitive ovarian cancer, it seems possible that this would enrich for patients likely to respond to a PARP inhibitor; at present, a similar strategy for enriching breast cancer patients by success of prior platinum salt response has not been extensively tested.

3. Resistance to PARP inhibitors

Several mechanisms by which cells can become resistant to PARP inhibitor cytotoxicity have been reported in the literature [15, 53, 56-60]. However, so far there is convincing clinical evidence for only one of these: secondary mutation of the original mutation causing the HR defect. These are often referred to as reversion mutations, although this term strictly refers to a mutation that restores the sequence to its unmutated state. More commonly, a compensating frameshift mutation is observed that restores the open reading frame of the gene; this does not necessarily result in the original nucleotide or protein sequence but can restore sufficient function to overcome the HR defect [10].

This mechanism of PARPi resistance was first described in pancreatic tumour cell lines with *BRCA2* mutation that were exposed to PARPi until resistant clones were isolated [53]. These mutant clones were also resistant to cisplatin. Secondary mutations of *BRCA1* and *BRCA2* that restored the reading frame of the respective gene were also observed in cell lines and ovarian cancers that had become cisplatin resistant [20, 54, 55]. A later report suggested that these mutations in cisplatin resistant tumours also caused cross-resistance to PARPi [61]. A *BRCA2* secondary mutation was also found in a tumour from a PARPi resistant male breast cancer patient [52]. More recently, secondary mutations have been found in several other tumour suppressor genes causing HR defects and BRCAness in ovarian cancer - *RAD51C, RAD51D* and *BRCA1* - suggesting that the principle of restoring function of the gene behind the original HR defect is a general feature of clinical PARPi resistance [19]. Circulating tumour DNA (ctDNA) analysis has identified secondary mutations in ovarian (in *BRCA1* and *RAD51C*), breast and prostate cancer patients (*PALB2* and *BRCA2* secondary mutations; [62-64]). This is significant as it raises the possibility of easily monitoring patients for the emergence of these resistance-causing mutations in the course of their treatment.

As PARP inhibitors move from being used in clinical trials in advanced disease to more routine use earlier in treatment, the development of

resistance mechanisms should be monitored. The propensity and consequence of reversion is likely to depend on exactly which pathogenic *BRCA*ness mutation is present. As more cases of reversion are documented, other mechanisms of resistance may become apparent. The recent ctDNA studies demonstrate that multiple secondary mutations that restore the reading frame can occur in the same patient; there may also be mutation events in other genes present that were not observed in these studies. *BRCA* reversions were not detected in all patients in these studies (for example, 2/5 *BRCA* mutant breast patients had a detectable reversion – in both cases this was a germline *BRCA2* mutation [62]), so other mechanisms may be at work in some cases. Different mechanisms of acquired resistance may result in different secondary drug sensitivities and thus be important in determining the choice of future therapy. For example, Wee1 inhibitors may be effective in *BRCA* mutant tumours with secondary mutations that restore HR [65].

4. PARP inhibitor combination therapy in breast cancer

Although there is ample evidence of the activity of PARPi in *BRCA* mutant cancers from single agent trials, it is clear that resistance eventually emerges in many cases. Management of this resistance will be key to obtaining prolonged responses when using PARPi. An appropriate combination therapy could be effective if it could be designed to target resistant clones as they emerge. Furthermore, as well as providing an approach to targeting PARPi resistant tumour subclones, combination approaches involving PARPi could also be used to extend the utility of PARPi beyond those patients with BRCA gene mutations.

4.1 Potentiating the molecular effects of PARP inhibitors

One potential strategy to enhance the therapeutic effect of PARPi and thus potentially expand their use to patients without BRCA gene mutations is to use them in combination with DNA damaging agents. Several classes of genotoxic DNA damaging agents, some of which are used clinically, are known to synergise with PARP inhibitors in cell line studies. These include alkylating agents, topoisomerase I inhibitors and ionizing radiation [66, 67].

4.1.1 DNA damaging agents

Temozolomide is the prototypical DNA alkylating agent that causes extreme sensitisation to PARP inhibitors. Alkylation damage to the bases of DNA is removed by damage specific glycosylases, eventually forming single stranded breaks that are detected and bound by PARP1. Agents that increase the number of SSBs increase the amount of cellular damage that needs to be processed by PARP1-mediated repair, as well as providing substrates for cytotoxic PARP1 trapping. Some trials have been carried out using temozolomide in combination with PARPi; however these combinations are poorly tolerated [68]. In the recent BROCADE trial (see below), the response rate in the veliparib/temozolomide group was also inferior to veliparib/carboplatin/paclitaxel (ORR 28.6% c.f. 61.3%; [69]).

Topoisomerase I inhibitors also synergise with PARPi, but by a slightly different mechanism. Here, PARP catalytic activity is required to remove trapped topoisomerase I-DNA complexes [70]. Again, in this case the potentiation is unlikely to be specific to HR-deficient tumour cells, although cells lacking other pathways of topoisomerase I clearance may be differentially sensitive. A phase I trial of topotecan in combination with veliparib observed high toxicity, necessitating the use of very low doses of both agents [71].

Some studies have also been carried out using PARP inhibitors in combination with platinum salts. Rather than causing SSBs, platinum-induced ICLs stall the passage of replication forks, resulting in collapse and single-ended double strand break structures. These are not as likely to induce PARP trapping to the same extent as SSBs, and the combinatorial effects of PARPi plus platinum are probably additive rather than synergistic [70]. However, a phase II trial in platinum sensitive ovarian cancer found a significant PFS increase when olaparib was added to a paclitaxel/carboplatin chemotherapy regimen, and also continued as a maintenance therapy [72]. Toxicity prevents continuous treatment at normal single agent doses of PARPi, resulting in the

use of intermittent dosing, perhaps reflecting the somewhat overlapping actions of these agents [73].

4.1.2 PARP inhibitors with platinum in breast cancer – BROCADE and Brightness

The BROCADE trial [69, 74] is a randomised phase II trial assessing the addition of veliparib to either carboplatin and paclitaxel combination therapy, or temozolomide, an alkylating agent, in locally recurrent or metastatic breast cancer with deleterious germline *BRCA1* or *BRCA2* mutation. HER2+ patients were excluded, and around 40% of patients had triple negative breast cancer (TNBC). Patients that had received prior platinum or PARPi therapy were also excluded. The dose of veliparib used (120 mg twice daily) is lower than would typically be used in single agent studies - this reflects the slightly different hypothesis being tested in this trial: that veliparib can potentiate the effects of DNA damaging chemotherapy in HR deficient cancer. In contrast to the ovarian trials described above, the BROCADE study did not show an increase in adverse effects when PARPi was combined with carboplatin, but also did not use full single agent PARPi MTD and used a similar intermittent schedule (PARPi given only on days 1-7 of the three-week carboplatin cycle). BROCADE also used veliparib rather than olaparib, therefore another possible explanation for these differences is that the increased toxicity in combination is specific to inhibitors with stronger trapping activity.

Patients in the BROCADE trial were randomised evenly to one of three groups: veliparib/carboplatin/paclitaxel, placebo/carboplatin/paclitaxel or veliparib/temozolomide. There was a significant increase in objective response rate (ORR) in the veliparib group compared to placebo (77.8% compared to 61.3%), but no significant difference in progression-free survival [69]. A larger phase III trial (BROCADE 3, NCT02163694) is now underway with PFS as the primary endpoint. The ORR observed in BROCADE (phase II) in both carboplatin/paclitaxel groups (with veliparib or placebo) is high for this patient population, likely due to activity of carboplatin in *BRCA* mutant

cancers. An analysis of data from the I-SPY2 trial also found a veliparib/carboplatin response rate of 75% in patients with a *BRCA*ness signature (Mammaprint High1/High2 +PARPi7, [75]) compared to 35% in patients lacking one of these signatures [76].

A related study, the “Brightness” trial (NCT02032277), used a similar randomisation setup to BROCADE to study the addition of veliparib and/or platinum to standard chemotherapy in the neoadjuvant setting in early TNBC. This is notable for being the only completed randomised phase III trial using a PARP inhibitor in early breast cancer. However, the addition of veliparib to carboplatin and paclitaxel (followed by standard doxorubicin and cyclophosphamide chemotherapy) did not increase the pCR rate beyond that achieved by adding carboplatin alone to paclitaxel [77]. This suggests that the activity in these patients is primarily due to carboplatin. These patients were not selected by *BRCA* mutation status, so it will be interesting to see whether the activity of veliparib beyond carboplatin is maintained in the BROCADE phase III trial where patients will all have a deleterious *BRCA* mutation. In early-stage breast cancer, a phase II trial investigating the addition of rucaparib to cisplatin prior to surgery (following standard neoadjuvant chemotherapy) is also underway; this trial will include TNBC patients regardless of *BRCA* status, as well as ER/PR-positive patients with known *BRCA* mutations (NCT01074970).

The PARTNER trial, currently underway, will assess paclitaxel/carboplatin with and without olaparib in the neoadjuvant setting in triple negative and/or gBRCA mutant breast cancer (NCT03150576).

4.1.3 Potentiation: activity and toxicity

The main issue with the use of agents that directly potentiate PARPi cytotoxicity is that this effect may not be specific to the HR-deficient tumour cells. Accordingly, the potentiating agent is likely to also worsen some of the on-target side effects of PARP inhibitors, and potentiating agents may not affect the "therapeutic window" - the relative difference in drug concentration

required to kill tumour and normal cells - even though the addition of a potentiating agent means that lower concentrations of PARPi are required. Since extremely potent PARPi are now available, the use of potentiating agents may not be necessary to achieve a therapeutic effect.

One exception to this principle of potentiating agents also causing increased toxicity is the use of PARPi as radiosensitisers. Although PARPi also sensitise non-tumour cells to ionising radiation, radiation can be localised somewhat to the tumour cells so it may be possible to circumvent any increased toxicity. A phase I trial of olaparib in combination with radiotherapy is being carried out in inoperable breast cancer (NCT02227082).

One class of drugs that is of interest in potential PARPi combination treatment is ATR inhibitors. ATR is a kinase involved in the DNA damage response and in the control of HR [78]. *ATR* gene silencing sensitises cells to PARPi [16], likely due to its role in promoting replication fork stability. Many cancer cells show high levels of "replication stress", a phenotype characterised by an increased frequency of fork stalling and mitotic abnormalities arising from under-replication of DNA. There is preclinical evidence that ATR inhibition in tumours that have high levels of replicative stress can sensitise cells to killing by PARP inhibitors, cisplatin, topotecan or gemcitabine [79]. Interestingly PARPi sensitisation with ATR inhibition was also seen in *BRCA1* depleted or *BRCA2* mutant cells beyond the PARPi sensitivity caused by the loss of HR function. It is possible that the use of ATR inhibitors will sensitise non-*BRCAm* TNBC, which may have BRCAness phenotypes with less severe HR defects and/or high levels of replicative stress, to killing by PARPi. There may also be single agent activity, as ATR inhibitors have also been shown to be selectively toxic to cancer cells that have activated the alternative lengthening of telomeres (ALT) pathway [80]. Clinical trials to test these hypotheses are currently being designed.

A better approach to combinations with PARPi might be to target other pathways, besides replication stress and SSB repair, that are still essential in the HR-deficient tumour cells, thus preserving the "therapeutic window"

between tumour and normal cells. These targets would not necessarily have to be based on the HR deficiency. Such agents would also likely have a different side effect profile, and perhaps be better tolerated. Orthogonal targets may also present an opportunity to target emerging PARPi resistance. Restoration of HR via a secondary *BRCA* mutation, for example, might still leave the cells with other defects related to their history of defective HR - not least the copy number variation and aneuploidy that is typical of *BRCA* defective cancer genomes. Targeting such an aspect of the tumour cells with an appropriate combination may result in a greater therapeutic effect that is still specific to the tumour cells. For example, recent work suggests that despite PARPi resistance, *BRCA1* mutant breast tumour cells with reversion mutations still retain sensitivity to inhibition of the WEE1 mitotic checkpoint kinase, probably because of their p53 defect and extensive genomic rearrangements [65]. Although PARP inhibitor treatment enhances the clonal expansion of *BRCA1* revertant clones in heterogeneous *in vitro* cell cultures and tumour xenografts, treatment with the clinical WEE1 inhibitor AZD1775 suppresses this; this might suggest that periodic WEE1 inhibitor treatment, used either in combination with or subsequent to a PARP inhibitor could be more effective than PARPi treatment alone [65].

4.2 Combinations with orthogonal mechanisms of action

4.2.1 Endocrine therapy

Although *BRCA1* mutant breast cancers are predominantly hormone-receptor negative, a significant proportion of *BRCA2m* breast cancers (70%) express the estrogen receptor (ERα), the target of endocrine therapies such as tamoxifen [81]. In clinical trials in advanced breast cancer, patients have disease that has progressed on prior therapy, including endocrine therapy. For example, in the OlympiAD study, 50% of patients were ERα or Progesterone Receptor (PR) positive. In a subgroup analysis of the OlympiAD study, hormone receptor positivity was associated with a poorer outcome compared to TNBC in terms of progression-free survival (HR 0.82 compared to 0.43). Interestingly, in the subgroup analyses of the EMBRACA trial,

hormone receptor-positive patients appeared to have a good outcome, similar to the TNBC subgroup (HR = 0.47). If confirmed this would be an important difference between olaparib and talazoparib, although the basis for such a difference is unclear. As more trials of PARPi in the early stages of disease treatment are completed, there may be an opportunity to combine PARPi with endocrine therapy in the adjuvant setting for *BRCAm* tumours that are ERα or PR positive. Since endocrine therapy would be expected to have an orthogonal mechanism of action to the PARPi, these could potentially be combined without leading to unacceptable toxicity or even mechanisms of cross resistance.

4.2.2 Immunotherapies

As in many areas of oncology, there is substantial interest in whether immune checkpoint inhibitors (e.g., anti-CTLA4, anti-PD-1 or anti-PD-L1 antibodies) may be effective in breast cancers and/or in combination with PARP inhibitors. In terms of rationalising why DNA repair inhibitors should be combined with immunotherapies, several arguments have been proposed. Some immunotherapies rely upon tumours expressing neo antigens that are recognised as non-self by the immune system. Such tumours depend critically on evading detection by the immune system, for example via activation of the PD-1/PD-L1 immune checkpoint, and thus are particularly vulnerable to blocking of this interaction by therapeutic antibodies. This may explain why many of the impressive immunotherapy responses thus far in are tumours with high mutational loads: melanoma, non-small cell lung cancer and mismatch repair deficient colorectal cancer [82-84].

A study in high grade serous ovarian cancer suggested that *BRCA1/2* mutation status or functional HR deficiency is associated with a higher predicted neoantigen load [85]. There was also a prognostic advantage of high neo-antigen load that was independent of *BRCA* mutation status. As such, one proposal is that an elevated neoantigen load in *BRCA* mutant tumours could enhance immunotherapy responses.

PARP inhibitors ultimately kill HR deficient cells by several rounds of cell division in the presence of persistent DNA damage [8, 86]. Such cell death could, in principle, result in shedding of damaged DNA and thus be immunogenic [87]. This is another reason to think that immunotherapy approaches may synergise with PARPi induced tumour cell death. This might also preserve the therapeutic window, as the PARPi cell death should also be specific to HR-deficient cells in this case.

PARPi treatment may also affect the immune system independently of its effects on DNA repair. Investigation of these effects is still at an early stage, and there are reports of different effects in different model systems. A recent report has shown that PD-L1 expression levels in breast cell lines are upregulated in response to PARP inhibitor exposure [88]. This attenuates T-cell killing of PARPi exposed cells, which can be restored by anti-PD-L1 exposure. If this effect occurs in tumours, this could provide another mechanism by which combination of PARPi with agents targeting the immune checkpoint. A syngeneic mouse study using talazoparib has been shown to promote immune cell infiltration in the tumour microenvironment [89]. Finally, a study has shown synergy between anti-CTLA4 and veliparib in the same syngeneic mouse ovarian cancer model [90].

Several trials are underway in breast cancer to test whether addition of immunotherapies provide an advantage over single agent PARPi. These are listed in Table 2.

Trial	Drugs	Patient population	Clinicaltrials.gov ID
MEDIOLA (ph. I/II)	Durvalumab + olaparib and/or cediranib	gBRCA, HER2-negative TNBC (and other solid tumours)	NCT02484404
DORA (ph. II)	Olaparib +/– durvalumab	Platinum sensitive TNBC	NCT03167619
TOPACIO (ph. I/II)	Niraparib + pembrolizumab	Advanced/metastatic TNBC	NCT02657889
NCT02849496 (ph. II)	Atezolizumab and veliparib (alone or in combination)	HR-deficient TNBC	NCT02849496

Table 2. Clinical trials studying the effect of PARPi in combination with immunotherapy drugs in breast cancer patients.

5. Ongoing breast trials

At the time of writing there are 41 ongoing studies assessing PARP inhibitors in breast cancer (clinicaltrials.gov). Some of these are combination therapy trials, referred to above, while other are assessing new PARP inhibitors. As well as OlympiAD and EMBRACA, there are other phase III trials underway that have reached their final data collection point for primary endpoint and for which results can be expected soon (Figure 2). These include BRAVO (niraparib compared to physician's choice chemotherapy, germline BRCA; NCT01905592) and BROCADE 3 (NCT02163694), assessing paclitaxel/carboplatin with or without veliparib.

The question of whether the unique PARP-trapping properties of talazoparib translate into better outcomes for patients, or just a reduced maximum tolerated dose (MTD) compared to other PARPi, has been partly addressed by the recent results of OlympiAD (assessing olaparib) and EMBRACA (assessing talazoparib). Indeed, the dose of talazoparib used is much lower (1

mg daily in EMBRACA) than other PARP inhibitors (typically in the hundreds of mg). Both of these trials showed a similar scale of benefit for their respective PARP inhibitor over chemotherapy. Talazoparib did have a higher relative frequency of adverse effects relative to chemotherapy when compared with to the OlympiAD data, but still was well-tolerated compared to chemotherapy, and both trials reported significant improvements in their respective quality of life measures with PARPi compared to chemotherapy. It is difficult to make a direct comparison of these data between these two trials but it seems that the drugs have broadly similar effects despite their differing trapping properties. Trapping potency could affect certain combinations with DNA damaging agents, however [70]. There are other subtle differences between the different PARP inhibitors that could potentially affect outcomes [91], but a trial to compare these directly seems unlikely at this point.

Another interesting question is whether PARP inhibitors will be of benefit when used earlier in the course of the disease. Most trials so far have been in advanced breast cancer. The OlympiA (NCT02032823) and Neo-Olympia trials aim to assess olaparib in the adjuvant and neoadjuvant setting respectively. Another recent development has been two "window" studies in which early stage breast cancer patients are treated with PARP inhibitors prior to surgery and monitored for evidence of a response. One pilot study observed a decrease in tumour volume in all *BRCA* mutant patients (n = 13) treated with talazoparib and has now progressed to a larger study [92]. The RIO study (ISRCTN92154110) will also apply this strategy using rucaparib in TNBC.

6. Expert Commentary

With the publication of encouraging phase III results demonstrating PFS benefit from the OlympiAD and EMBRACA trials, and other phase III trials due to report in the near future, the comparative drought of clinical data regarding PARPi response in breast cancer compared to ovarian cancer is coming to an end. It is likely that further encouraging results from these trials will result in breast cancer approvals for other PARP inhibitors. The task will then be to

optimise PARPi therapy, in terms of combination, stage and scheduling to achieve maximum benefit and manage the emergence of resistance.

7. Five-year view

Given the wealth of phase III clinical trials that are reaching their endpoints, the next five years are likely to see further practice-changing approvals of PARP inhibitors for some of these indications. The EMBRACA trial results suggest that talazoparib will receive a similar approval to olaparib, and given that niraparib is already approved in ovarian cancer it is likely that this drug will also find utility in breast cancer, subject to results of the BRAVO trial. The overall evidence for PARPi activity in advanced gBRCAm breast cancer is now unequivocal, and we may soon see the use of PARPi in early disease, based on the upcoming window and neoadjuvant studies. There is also possibility of renewed interest in preventative strategies for *BRCA* carriers, given their high risk and the continuing good results showing long-lasting protection from ER-positive disease in high-risk individuals afforded by periods of prophylactic endocrine therapy [93, 94]. There are legitimate concerns about the potential for PARP inhibitors to induce DNA damage, but the combination of more data from advanced cancer patients receiving long term treatment and data from window studies that shed light on the response of early stage disease may lead to reconsideration of the chemoprevention approach.

8. Key Issues

- Recent data from phase III trials of single agent PARP inhibitors in advanced g*BRCAm* breast cancer has resolved doubts about the activity of PARP inhibitors in this disease.
- The OlympiAD trial data have supported the first regulatory approval of a PARP inhibitor, olaparib, for breast cancer. Olaparib treated patients had a median progression-free survival of 7.0 months, compared to 4.2 months in chemotherapy-treated patients (Hazard Ratio (HR) = 0.58).

There was no significant difference in overall survival, although the study was not designed to detect this.

- Similar trials for other PARP inhibitors are reaching completion for talazoparib (EMBRACA trial, recently reported) and niraparib (BRAVO trial). Since the designs of these phase III trials are similar, they may allow hypotheses about the relative merits of the different drugs to be formulated and tested.
- Although a number of potential PARPi resistance mechanisms have been described in laboratory studies, the only confirmed mechanism of clinical resistance described so far has been through secondary mutations that restore function of the mutated homologous recombination gene.
- Data from various combination therapy trials are likely to be key to expanding the utility of PARP inhibitors beyond g*BRCAm* and/or dealing with single agent resistance, which has already been observed.
- PARP inhibitors and platinum salts have overlapping mechanisms of action and resistance mechanisms to some extent. It will be necessary to interpret PARP inhibitor trial results in the context of ongoing trials of platinum therapy.

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To highlight:
Robson et al ** Results of the OlympiAD trial, demonstrating a progression-free survival benefit compared to chemotherapy and leading to the approval of olaparib for gBRCAm metastatic breast cancer.
Coleman et al ** ARIEL3 trial in ovarian cancer, which made use of an LOH signature to select patients.
Han et al ** Phase II BROCADE trial suggesting a high response rate to carboplatin, with a minor additional benefit from veliparib

Murai (2012) * This study describes the correlation between trapping and cytotoxicity for a range of clinical PARP inhibitors.
Khondrashova *
Goodall *
Quigley *
Weigelt * Studies demonstrating reversion of HR defects as a resistance mechanism to PARPi and/or platinum.

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