Expert Reviews

PARP inhibition and synthetic lethality in ovarian cancer

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Ovarian cancer is the leading cause of gynecologic cancer death in women. Our understanding of the treatment of ovarian cancer has evolved over the last decade, with the use neo-adjuvant chemotherapy, combined intravenous-intraperitoneal (IV-IP) chemotherapy, as well as dose dense paclitaxel. Despite significant improvements in overall survival, the majority of patients succumb to recurrent chemotherapy resistant disease. Given the above, an emphasis has been placed on exploring alternate therapeutics. Recent research efforts have improved our understanding of the molecular biology of ovarian cancer and novel targeted treatment strategies have emerged. With the discovery of BRCA1 and BRCA2 gene mutations, and a more comprehensive assessment of heredity ovarian cancer syndrome, targeted interventions exploiting this biologic susceptibility have emerged. To date, the most studied of these have been PARP inhibitors. The purpose of this review will be to discuss PARP inhibition in advanced stage ovarian cancer, highlighting recent scientific advancements.

Keywords: cancer • inhibition • lethality • ovarian • PARP

Ovarian cancer continues to be the leading cause of gynecologic cancer death in the USA. In 2014, there will be an estimated 21,980 new cases diagnosed with an anticipated 14,270 deaths [1]. Despite advancements in surgical cytoreduction and adjuvant cytotoxic chemotherapy, limited survival gains have been achieved over the past decade. Specifically, therapeutic paradigms incorporating intravenous plus intraperitoneal chemotherapy, hyperthermic intraperitoneal chemotherapy and a weekly dose-dense schedule of paclitaxel have been explored with variable success [2–4].

As our understanding of tumor biology evolves, molecular pathways predicting response to targeted novel agents have drawn a significant amount of attention. These pathways are heterogeneous, however, and built-in redundancy has limited single-agent success. To date, the only biologic agent examined in patients with gynecologic cancer exhibiting an overall survival (OS) advantage is the antiangiogenic agent bevacizumab in a population of patients suffering from advanced stage, recurrent or progressive cervical cancer [5]. This benchmark has yet to be achieved in the ovarian cancer arena.

In an effort to identify molecular aberrations potentially contributing to the pathogenesis of ovarian cancer, The Cancer Genome Atlas was completed, analyzing mRNA expression, miRNA expression, promoter methylation and DNA copy number in 489 high-grade serous ovarian adenocarcinomas and the DNA sequences of exons from coding genes in 316 of these tumors [6]. The authors identified *TP53* mutations in 96% of tumor samples. Additionally, statistically recurrent somatic mutations were identified in nine further genes including *NF1*, *BRCA1*, *BRCA2*, *RB1* and *CDK12* [6]. Genomic disarray was prevalent among cancer subtypes with 113 significant focal DNA copy number aberrations.

Importantly, pathway analyses suggested that homologous recombination (HR) was defective in about half of the tumors analyzed. Over 20% of high-grade serous ovarian cancer specimens examined exhibited germline or somatic mutations in *BRCA1* and *BRCA2*. An additional 11% of samples lost BRCA1 expression through DNA hypermethylation, with the epigenetic silencing of *BRCA1* being mutually exclusive of *BRCA1/2* mutations. The importance of the above findings rests on our prior understanding of *BRCA* function and dysfunction.

The Fanconi anemia BRCA pathway

Fanconi anemia (FA) is an autosomal recessive disease that is defined by bone marrow failure and cancer susceptibility. Named after Guido

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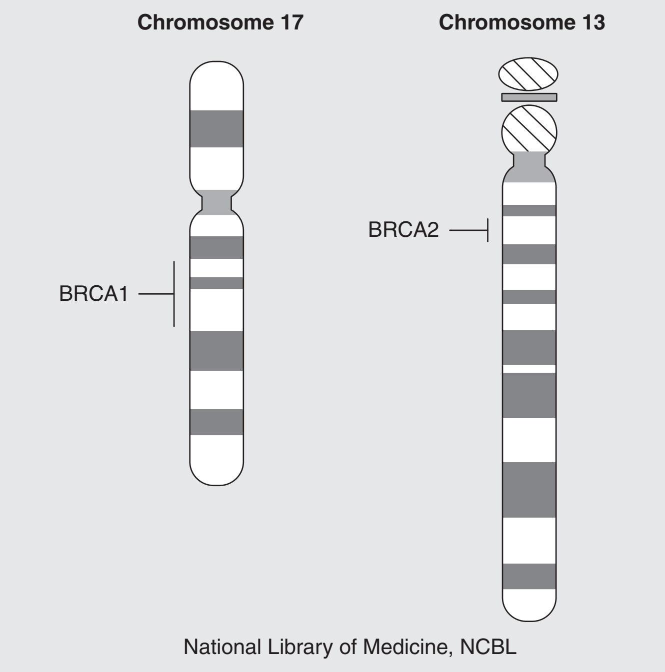

Figure 1. The *BRCA1* and *BRCA2* genes on chromosomes 17 and 13, respectively.
BRCA1: Breast cancer 1, early onset; BRCA2: Breast cancer 2, early onset.
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Fanconi (January 1892 to October 1979), a renowned pediatrician who recognized the syndrome's characteristic short stature and hyperpigmentation, FA is a genetic condition resulting in pancytopenia, often leading to death at a young age secondary to infectious morbidity [7,8].

The clinical features of FA include skeletal anomalies, skin pigmentation, cardiac, renal and gastrointestinal pathology and a predisposition to many types of cancer [9]. Patients with FA are sensitive to DNA cross-links and ionizing radiation secondary to defects in DNA damage repair with subsequent genomic instability. The pathway itself is critical in the modulation of DNA repair by HR.

FA, analogous to similar inherited cancer susceptibility syndromes, has provided insight into the genetic basis of cancer [9]. The FA pathway comprises five FA proteins (A, C, E, F and G) that regulate activation via monoubiquitylation of FANCD2. Activated FANCD2 subsequently targets BRCA1 nuclear foci. Patients with genetic defects in this pathway, including *BRCA1* and *BRCA2,* suffer from chromosomal instability, predisposing to cancer, while simultaneously sensitizing tumors to cytotoxic DNA alkylating agents.

In a series of elegant experiments, the relationship between FANCD1 and BRCA1/2 was elucidated. Garcia-Higuera *et al.* determined that the activated FANCD2 protein colocalized with the breast cancer susceptibility protein, BRCA1, in ionizing radiation-induced foci and in synaptonemal complexes of meiotic chromosomes [10]. The FANCD2 protein, therefore, provided the missing link between the FA protein complex and the cellular BRCA1 repair machinery. Additionally, Howlett *et al.* showed that cell lines derived from FA-B and FA-D1 patients had biallelic mutations in *BRCA2* and expressed truncated BRCA2 proteins. Functional complementation of FA-D1 fibroblasts with wild-type *BRCA2* complementary DNA restored mitomycin-C resistance and linked the six cloned FA genes with *BRCA1* and *BRCA2* in a common pathway [11].

BRCA1 & BRCA2

Germline *BRCA1* and *BRCA2* mutations have long been recognized as conferring the greatest risk for both breast and ovarian cancer. The *BRCA1* and *BRCA2* genes are located on chromosomes 17 and 13, respectively (FIGURE 1). These genes are essential for cellular development with pivotal roles in genomic stability. The absence of either *BRCA1* or *BRCA2* results in chromosomal rearrangements and is lethal in embryonic development [12].

Functional *BRCA* genes are required for error-free HR. While HR is not the only mechanism available for DNA damage repair, the alternative processes, nonhomologous end joining (NHEJ) and single-strand annealing (SSA), are error prone and frequently result in gross chromosomal rearrangements [13,14]. In fact, in the synthesis and G2 phases of the cell cycle, HR predominates as the mechanism of repair and the BRCA proteins are at maximal expression [12].

Molecular studies performed in *BRCA1*-deficient mouse embryonic stem (ES) cells showed impaired repair of chromosomal double-stranded breaks (DSBs) by HR [15]. The relative frequencies of homologous and nonhomologous DNA integration and DSB repair were also altered. These results demonstrated a caretaker role for *BRCA1* in preserving genomic integrity by promoting HR and limiting mutagenic nonhomologous repair processes, including both NHEJ and SSA. Furthermore, the loss of *BRCA2* resulted in misrepair of chromosomal DSB occurring between repeated sequences by stimulating the use of error-prone recombination pathways [16].

The impact of germline *BRCA1* and *BRCA2* mutations on oncologic outcome in patients with ovarian cancer began to emerge nearly two decades ago. Boyd *et al.* explored progression-free survival (PFS) and OS in a retrospective cohort of Jewish patients with advanced stage epithelial ovarian cancer and both mutant and wild-type *BRCA* alleles [17]. From the 189 patients who identified themselves as Jewish, 88 hereditary cases were identified with the presence of a germline founder mutation in *BRCA1* or *BRCA2*. The remaining 101 cases from the same series not associated with a *BRCA* mutation and two additional groups with ovarian cancer from clinical trials (Gynecologic Oncology Group protocols 52 and 111) were included for comparison. The groups were balanced with respect to clinicopathologic characteristics. However, the *BRCA* mutation group had a longer disease-free interval following primary chemotherapy in comparison with the nonhereditary group with a median time to recurrence of 14 and 7 months, respectively ($p < 0.001$) [17]. Additionally, those with hereditary cancers had improved survival compared with the nonhereditary group ($p = 0.004$).

Additional investigators confirmed the above findings by showing that patients with *BRCA* mutation-related (hereditary) epithelial ovarian cancer exhibited improved PFS, OS as well as increased sensitivity to platinum agents in the adjuvant setting [18–24].

BRCA & poly-ADP ribose polymerase interplay & the concept of synthetic lethality

DNA damage can involve single-stranded DNA break or DSB. A total of six separate DNA repair pathways have been identified, playing a critical role in DNA integrity and cell survival. Currently, the major DNA repair pathways include mismatch repair, base excision repair (BER), nucleotide excision repair, DSB recombinatorial repair and NHEJ [25]. These pathways respond to DNA damage induced by ultraviolet light, cross-linking agents, ionizing radiation, alkylating agents, radiotherapy and chemotherapeutic agents with redundant and interdependent roles. Lesions affecting only one DNA strand rely on the use of the complementary strand for repair utilizing the BER, nucleotide excision repair and mismatch repair pathways. Conversely, DSBs are more problematic as a complementary strand is not available as a template for repair [26].

As previously discussed, both *BRCA1* and *BRCA2* functions are required for accurate HR, a high fidelity repair pathway. More recently, the role of poly-ADP ribose polymerases (PARPs) in the repair of single-stranded DNA breaks has emerged. The most extensively studied of the PARPs is PARP-1, which utilizes nicotinamide adenine dinucleotide to synthesize ADP-ribose polymers on nuclear proteins associated with chromatin or on itself [12]. PARP-1 exhibits both direct and indirect DNA repair activity. The indirect component involves x-ray repair cross-complementing protein 1 recruitment and chromatin loosening that allows the repair enzyme access to portions of damaged DNA [27]. The direct component of PARP-1 function involves BER, polymerization via DNA polymerase beta and ligation mediated by DNA ligase III [28,29]. The importance of PARP-1 in BER was demonstrated in knockdown experiments where PARP-1-deficient cells exhibited increased RAD51 foci, signaling DSB repair [30].

Ultimately, PARP-1 deficiency results in a failure to repair single-stranded DNA breaks, which translate into DSB at the replication fork when left unrepaired (Figure 2) [28,29]. Under normal conditions, these lesions would be repaired using high fidelity, BRCA-dependent HR mechanisms. However, in BRCA-deficient cells, these DSBs are repaired using mutagenic nonhomologous repair processes such as NHEJ and SSA, resulting in chromosomal instability, cell cycle arrest and apoptosis.

Mice lacking either *BRCA1* or *BRCA2* exhibit embryonic lethality, indicating an essential function in cellular development. Conversely, PAPR-1 null mice remain viable and fertile, likely due to the presence of high fidelity HR pathways. Given the above, the mechanistic advantage of PARP inhibition is greatest in patients with heterozygous *BRCA1/2* mutations who subsequently undergo a second somatic loss in the target tissue of interest [31]. This concept of synthetic lethality implies that tumor tissues evolve into a *BRCA* null state with defective HR, resulting in enhanced sensitivity to PARP inhibition. Conferring a potential therapeutic benefit, cell death is limited to homozygous target tissues (i.e., tumor), limiting toxicity to normal neighboring cells.

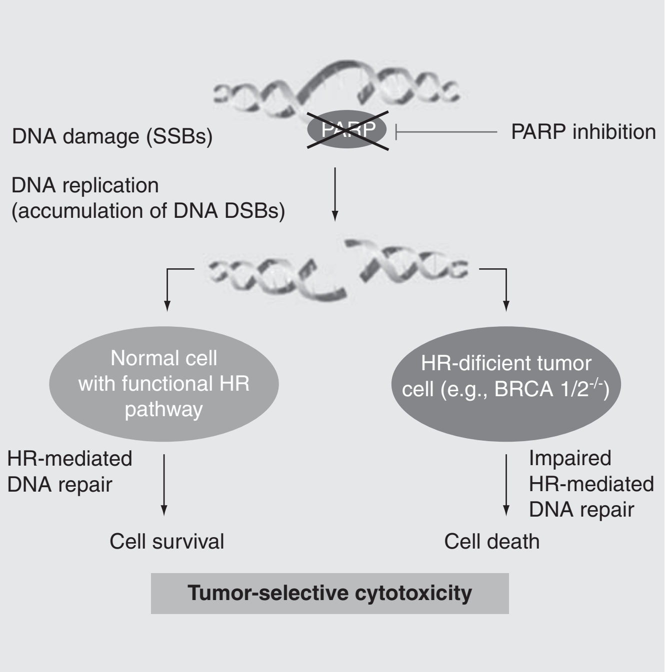

Figure 2. PARP-1 deficiency results in a failure to repair single-stranded DNA breaks, which when left unrepaired, translate into DSB at the replication fork. Under normal conditions, these lesions would be repaired using high fidelity, BRCA-dependent, homologous recombination mechanisms. However, in *BRCA*-deficient cells, these DSBs are repaired using mutagenic nonhomologous repair processes, such as NHEJ and SSA, resulting in chromosomal instability, cell cycle arrest and apoptosis.

DSB: Double-stranded DNA breaks; NHEJ: Nonhomologous end joining; PARP: Poly-ADP ribose polymerase; SSA: Single-strand annealing.

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PARP inhibitor activity: proof of concept

In a series of landmark publications, the clinical utility of PARP inhibition in *BRCA*-deficient cell lines was described [32–34]. Using an ES cell model, Farmer *et al.* demonstrated a clear reduction in clonogenic survival of *BRCA1* and *BRCA2*-deficient cells following PARP-1 siRNA plasmid transfection [33]. Furthermore, the chemical inhibitors of PARP activity (KU0058684 and KU005894) demonstrated 57- to 133-fold enhanced activity in cells lacking wild-type *BRCA1* and *BRCA2*. Notably, in the above model, none of the inhibitors exhibited selective effects in cells heterozygous for *BRCA1/2* mutations. On a molecular level, PARP inhibition resulted in DNA damage (triradial and quadriradial chromosomes), G2 cell cycle arrest and ultimately apoptosis. The *in vivo* efficacy

Table 1. Poly-ADP ribose polymerase inhibitors currently under development.

Company	Drug (PARP inhibitor)	Phase of investigation
AstraZenica	Olaparib	I II III
Abbott	Veliparib	I II
BioMarin	BMN673	I
Clovis	Rucaparib	I II III
TESARO	Niraparib	I III

PARP: Poly-ADP ribose polymerase.

of PARP inhibition was examined using ES cell teratocarcinomas transplanted into athymic mice. KU0058684 treatment severely inhibited the formation of tumors derived from *BRCA2*-deficient cells [33].

Analogously, Bryant *et al.* examined the impact of PARP inhibition on cell lines deficient in the HR proteins XRCC2 and XRCC3 [34]. The HR-deficient cell lines were sensitive to all PARP inhibitors. Furthermore, the PARP inhibitors NU1025 and AG14361 were profoundly cytotoxic at low concentrations in the *BRCA2*-deficient cell line V-C8. Using this same cell line, V-C8, investigators were able to demonstrate that *BRCA2*-deficient tumors in a xenograft animal model were exquisitely sensitive to treatment with PARP inhibition alone [34]. In combination, the findings above catalyzed the clinical investigation of PARP inhibitors as a safe and effective treatment of patients with defective HR.

The confirmation of the clinical link between PARP inhibition and BRCA mutation was first described in a prospective Phase I clinical trial examining the use of olaparib (AZD2281), a novel, potent, orally active PARP inhibitor in a population enriched in carriers of *BRCA1* or *BRCA2* mutations [35]. Sixty heavily pretreated patients with refractory solid tumors, 22 of whom were carriers of a *BRCA1* or *BRCA2* mutation, were enrolled in the study. Within the cohort, *BRCA* mutation carriers had a significant objective tumor response with a 47% partial response rate. Remarkably, of the nine patients with a partial response, eight had advanced stage recurrent epithelial ovarian cancer. No objective responses were observed in patients without known *BRCA* mutations. This trial also established the maximum tolerable dose of olaparib at 400 mg orally twice daily with only minimal adverse effects including fatigue and gastrointestinal toxicity. Three novel PARP inhibitors (niraparib, rucaparib, BMN673) have also been studied in prospective Phase I trials of heterogeneous patient populations (Table 1). With respect to the ovarian cancer cohorts, reported objective response rates (ORRs) ranged from 40 to 65% [36,37].

The concept of BRCAness

Given the contribution that *BRCA1* and *BRCA2* mutations make to hereditary cancer predisposition, it is surprising that these genes are only rarely inactivated by mutations in sporadic cancers [38]. However, following the identification of PARP inhibitors and the therapeutic concept of synthetic lethality, investigators began to identify *BRCA*-like molecular and clinical characteristics in various solid tumors. Using gene expression profiling, investigators were able to demonstrate similarities between *BRCA1* mutant familial breast cancers and sporadic basal-type breast cancer [39]. Additionally, the *BRCA* mutation-related ovarian cancers were commonly of high-grade serous histology and exhibited a uniform clinical behavior with high overall response rate to first-line platinum therapy, high response rates to platinum-based chemotherapy at recurrence, long disease-free intervals and improved OS [17,40]. Ultimately, the term BRCAness was created to describe this BRCA-like phenotype in sporadic ovarian cancers.

Further molecular studies identified epigenetic processes in the BRCA1/2-FA pathway, resulting in an analogous phenotypic expression. The aberrant methylation of the BRCA1 promoter has been described in 5–31% of sporadic ovarian cancers, while Fanconi F methylation and loss or reduction in FANCD2 translates into HR deficiency [38]. EMSY, a protein that leads to *BRCA* silencing, is amplified in up to 20% of high-grade serous ovarian cancer disrupting *BRCA2* participation in the DNA damage response [38]. The additional inactivation of *RAD51C* and the DNA damage sensory proteins, ATM and ATR, have been identified in 2–3% of sporadic ovarian cancer.

More recently, a gene expression profile of BRCAness that correlated with chemotherapy response and outcome was shown to be independently prognostic in patients with sporadic epithelial ovarian cancer [41]. Utilizing publicly available microarray data sets, the BRCAness profile accurately predicted platinum responsiveness in 8 out of 10 patient-derived tumor specimens and between PARP inhibitor sensitivity and resistance in four out of four Capan-1 clones. Additionally, in 70 patients with sporadic ovarian carcinoma, patients with the BRCA-like profile had improved disease-free survival (34 vs 15 months; log-rank $p = 0.013$) and OS (72 vs 41 months; log-rank $p = 0.006$) compared with patients with a non-BRCA-like profile. For the above reasons, the use of PARP inhibitors is not strictly limited to only those patients with germline *BRCA* mutations in current clinical trials.

PARP inhibition in ovarian cancer: the Phase II arena

Given promising preclinical data and the pronounced clinical effect identified in Phase I trials, investigation of PARP inhibition rapidly entered the Phase II arena in both single agent and combination studies. To date, all have been conducted in the recurrent setting (Table 2).

In one of the earlier studies, olaparib, at a dose of 400 mg twice daily, was administered to patients with advanced stage triple-negative breast cancer or high-grade serous and/or

Table 2. Phase II studies of poly-ADP ribose polymerase inhibitors in patients with ovarian cancer.

Study (year)	N	Drug dose and schedule	ORR	PFS	Grade 3/4 AEs	Ref.
Gelmon *et al.* (2011)	65	Olaparib 400 mg orally b.i.d.	41% in BRCAm 24% in BRCAwt	Not reported	Fatigue, nausea, emesis and decreased appetite	[42]
Audeh *et al.* (2010)	56	Olaparib 400 mg orally b.i.d. (n = 33) Olaparib 100 mg orally b.i.d. (n = 24)	33% in 400 mg arm 13% in the 100 mg arm	Not reported	Nausea, fatigue and anemia[†]	[43]
Kaye *et al.* (2012)	97	Olaparib 200 mg orally b.i.d. vs Olaparib 400 mg orally b.i.d. vs PLD 50 mg/m^2 every 28 days	25% in 200 mg arm 31% in the 400 mg arm 18% in the PLD arm	6.5 months 8.8 months 7.1 months[‡]	Nausea, fatigue, emesis, anemia[§]	[44]
Ledermann *et al.* (2013)	265	Olaparib 400 mg orally b.i.d. versus placebo	12% olaparib arm 4% placebo	8.8 vs 4.8 months (HR: 0.35; $p < 0.001$)	Nausea, fatigue, emesis, anemia	[46]
Coleman *et al.* (2014)	52	Veliparib 400 mg orally b.i.d.	Total confirmed responders: 26%	PFS 8.1 months OS 19 months	Nausea, emesis, neutropenia, thrombocytopenia	[47]

[†] Only reported in the 400 mg arm.
[‡] Nonsignificant HR 0.88 with respect to survival.
[§] Only in the olaparib arm.
AEs: Adverse events; b.i.d.: Two-times a day; BRCAm: BRCA1 or BRCA2 mutation carrier; BRCAwt: BRCA wild type; OS: Overall survival; ORR: Objective response rate; PFS: Progression-free survival; PLD: Pegylated liposomal doxorubicin.

undifferentiated ovarian cancer [42]. This Phase II, open-label, nonrandomized study stratified patients according to whether they had a *BRCA1* or *BRCA2* mutation or not. The primary endpoint was ORR by Response Evaluation Criteria in Solid Tumors (RECIST). Patients who had measurable lesions at baseline were included in the primary efficacy analysis. A total of 91 patients were enrolled between July 2008 and September 2009. Sixty-five had advanced stage recurrent ovarian cancer, of which 63 were evaluated for objective response as per RECIST. Confirmed objective responses were seen in 7 of 17 patients (41%; 95% CI: 22–64) with *BRCA1* or *BRCA2* mutations and 11 of 46 patients (24%; 95% CI: 14–38) without mutations. The most common adverse events were fatigue (70% of patients with ovarian cancer, 50% of patients with breast cancer), nausea (66 and 62%), vomiting (39 and 35%) and decreased appetite (36 and 27%). This was also the first clinical trial to show activity with olaparib monotherapy in a cohort of pretreated high-grade serous ovarian cancer patients without germline *BRAC1* or *BRCA2* mutations.

Audeh *et al.* conducted an international, multicenter, sequential cohort Phase II study of women with advanced stage recurrent ovarian cancer and confirmed genetic *BRCA1* or *BRCA2* mutations [43]. The first cohort of women (n = 33) was given continuous oral olaparib at the maximum tolerated dose of 400 mg twice daily and the second cohort (n = 24) was given continuous oral olaparib at 100 mg twice daily. The primary efficacy endpoint was ORR. The enrolled patients had received a median of three previous chemotherapy regimens (range 1–16). The ORR was 33% (95% CI: 20–51) in the 400 mg cohort and 13% (95% CI: 4–31) in the 100 mg cohort. Once again, the most frequent treatment-related grade 3/4 adverse events included nausea, fatigue and anemia and occurred only in the 400 mg cohort. This study once again supported the efficacy and tolerability of genetically targeted treatment with olaparib in *BRCA*-mutated advanced ovarian cancer.

Given the convincing single-agent activity of olaparib in the *BRCA*-mutated population, investigators looked to compare this targeted therapy with conventional cytotoxic chemotherapy. In a prospective Phase II clinical trial, patients with recurrent serous ovarian cancer (interval <12 months since prior platinum therapy) and confirmed *BRCA1* or *BRCA2* deficiency were assigned in a 1:1:1 ratio to olaparib 200 mg twice per day or 400 mg twice per day continuously or pegylated liposomal doxorubicin (PLD) 50 mg/m^2 intravenously every 28 days [44]. The primary efficacy endpoint was RECIST assessed PFS. Secondary endpoints included ORR and safety. A total of 97 patients were randomly assigned. Median PFS was 6.5 months (95% CI: 5.5–10.1 months), 8.8 months (95% CI: 5.4–9.2 months) and 7.1 months (95% CI: 3.7–10.7 months) for the olaparib 200 mg, olaparib 400 mg and PLD groups, respectively. There was no statistically significant difference in PFS (hazard ratio [HR]: 0.88; 95% CI: 0.51–1.56; p = 0.66) for combined olaparib doses versus PLD. RECIST-assessed ORRs were 25, 31 and 18% for olaparib 200 mg, olaparib

400 mg and PLD, respectively; differences were not statistically significant. The tolerability of both treatments was as expected with frequency of adverse events consistent with previously reported toxicity profiles. No significant differences in health-related quality of life were identified between treatment arms.

The largest Phase II study, completed by Ledermann *et al.*, examined the use of maintenance olaparib in a cohort of platinum-sensitive high-grade serous ovarian cancer patients, following a partial or complete response to their most recent line of platinum-based therapy (enrollment limited to ≤2 prior lines of therapy) [45]. This prospective double-blind, placebo-controlled trial randomly assigned 265 patients to receive olaparib, at a dose of 400 mg twice daily or placebo. The primary endpoint was PFS according to RECIST guidelines. Of the 265 patients who underwent randomization, 136 were assigned to the olaparib group and 129 to the placebo group. PFS was nearly double with olaparib than with placebo (8.4 vs 4.8 months from randomization on completion of chemotherapy; HR: 0.35; 95% CI: 0.25–0.49; p <0.001). The subgroup analyses of PFS showed that, regardless of subgroup, patients in the olaparib group had a lower risk of progression. Adverse events were more commonly reported in the olaparib group than in the placebo group and included nausea (68 vs 35%), fatigue (49 vs 38%), vomiting (32 vs 14%) and anemia (17 vs 5%) [45]. An interim analysis of OS (38% maturity) showed no significant difference between groups (HR: 0.94; 95% CI: 0.63–1.39; p = 0.75).

Since the completion of this trial, an additional interim survival analysis with 58% maturity was conducted with a dramatic HR of 0.18 (95% CI: 0.11–0.31) favoring the olaparib maintenance arm in the *BRCA* mutation population [46]. The median PFS was nearly three times greater in patients receiving olaparib relative to placebo, 11.2 versus 4.3 months, respectively. An OS advantage was not seen with olaparib (HR: 0.74; 34.9 vs 31.9 months) and may be attributable to crossover with 22.6% of the placebo arm receiving olaparib at the time of progression.

More recently, the results of Gynecologic Oncology Group protocol 280 were presented at the Society of Gynecologic Oncologists annual meeting in March of 2014 [47]. This prospective Phase II clinical trial investigated the efficacy of veliparib, a potent small-molecule inhibitor of PARP-1 and PARP-2, in women with documented *BRCA1* or *BRCA2* germline mutations in the setting of persistent or recurrent disease. Up to three prior treatment regimens were allowed, although prior PARP inhibition therapy was excluded. Veliparib was administered at 400 mg p.o. twice daily with up to two dose-level reductions for toxicity. One cycle was 28 days. Of 50 enrolled and eligible patients, 30 were platinum resistant and 20 were platinum sensitive. Thirty-six percent received three prior lines of chemotherapy. The median number of cycles administered was 5.5 (range: 1–16). There was one grade 4 thrombocytopenia. Grade 3 adverse events included fatigue (n = 3), nausea (n = 2), leukopenia (n = 1), neutropenia (n = 1), dehydration (n = 1) and elevation in liver enzymes (ALT: n = 1). The confirmed response rate was 26% (90% CI: 16–38%, complete response: 1; partial response: 12). The response rates in platinum-resistant and platinum-sensitive patients were 20 and 35%, respectively. The most common reason for treatment discontinuation was disease progression (46%). The median PFS was 8.11 months (90% CI: 5.45–8.77), and the proportion of patients event-free at 6 months was 44% [47]. These promising findings support the tolerability and clinical activity of veliparib in both platinum-sensitive and resistant populations, warranting further investigation.

PARP inhibition in ovarian cancer: the Phase III arena

As the efficacy and safety of PARP inhibition in patients with serous ovarian cancer and germline *BRCA* mutation were confirmed in Phase II studies, several prospective Phase III trials were designed and were opened for enrollment (FIGURE 3 & TABLE 3).

AstraZeneca has developed two separate prospective, randomized Phase III clinical trials investigating the use of olaparib in the upfront and recurrent setting. Study of olaparib in ovarian cancer (SOLO) 1 [48] is a double-blind, placebo-controlled, multicenter study evaluating the safety and efficacy of olaparib in patients with *BRCA*-mutated advanced ovarian cancer following a complete or partial response to first-line platinum-based chemotherapy. Patients will be randomized 2:1 (estimated enrollment: n = 344) to olaparib versus placebo. The primary endpoint is PFS and secondary endpoints include OS, time from randomization to second progression, safety and quality of life. Patients enrolled in this study must have a deleterious *BRCA1* or *BRCA2* mutation and have completed first-line platinum-based chemotherapy with a clinical complete or partial response. Both primary surgical cytoreduction and neoadjuvant chemotherapy are acceptable therapeutic algorithms.

The sister study, SOLO 2 [49], is a comparable trial examining olaparib in the recurrent setting. Patients with confirmed *BRCA1* or *BRCA2* deleterious mutation will be randomized 2:1 (estimated enrollment: n = 264) to olaparib versus placebo. Once again, the primary endpoint is PFS. All subjects must have received at least two prior lines of platinum-containing therapy prior to randomization with a documented complete or partial response on completion of the chemotherapy course immediately prior to randomization. Importantly, patients requiring therapeutic paracentesis during the final two cycles of their last chemotherapy regimen are excluded from enrollment.

Additionally, Tesaro, Inc. developed niraparib and has opened the niraparib ovarian (NOVA) protocol to enrollment [50]. In this double-blind, placebo-controlled, international Phase III trial, an estimated 360 patients with recurrent platinum-sensitive serous ovarian cancer will be randomized 2:1 to receive niraparib or placebo and will be continuously treated with placebo or 300 mg of niraparib until progression. The primary endpoint of this study is PFS. Secondary endpoints include patient-reported outcomes, chemotherapy-free interval length and OS. Importantly, two independent cohorts will be enrolled in the study, women with high-grade platinum-sensitive serous histology and no evidence of a deleterious *BRCA* mutation and subjects with a known *BRCA1* or *BRCA2* mutation. All patients are required to have received at least two

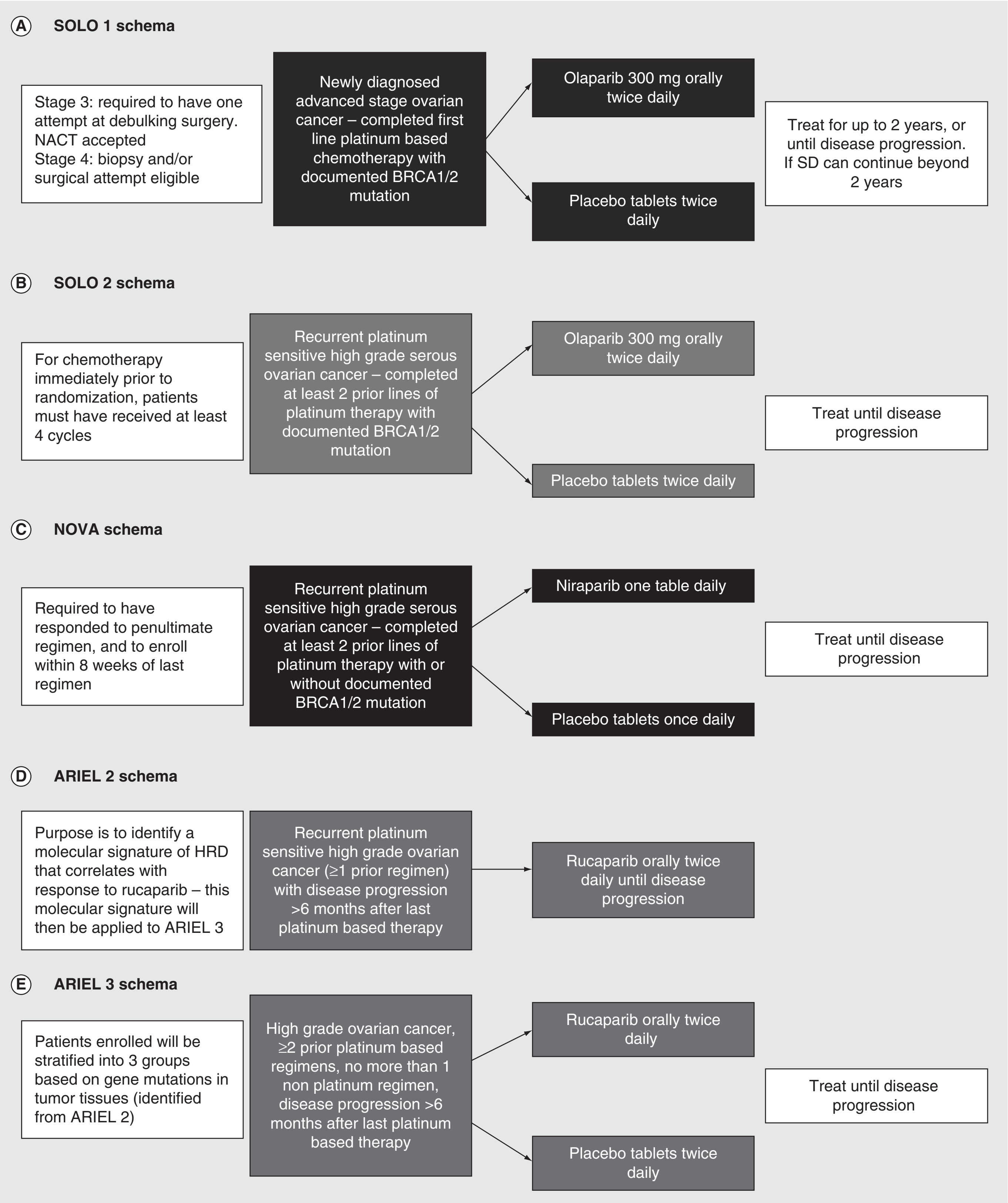

Figure 3. Schema of Phase II and Phase III trials exploring PARP inhibition in patients with ovarian cancer.
ARIEL: Assessment of Rucaparib In Ovarian CancEr TriaL; BRCA1: Breast cancer 1, early onset; BRCA2: Breast cancer 2, early onset; HRD: Homologous recombination deficiency; NOVA: Niraparib ovarian; PARP: Poly-ADP ribose polymerase; SOLO: Study of olaparib in ovarian cancer.

Table 3. Phase III trials examining poly-ADP ribose polymerase inhibitors in the treatment of ovarian cancer.

Trial	Disease setting	Agent	Ref.
SOLO 1	Following a complete or partial response to first-line platinum-based chemotherapy	Olaparib	[48]
SOLO 2	Platinum-sensitive recurrent ovarian cancer	Olaparib	[49]
NOVA	Platinum-sensitive recurrent ovarian cancer	Niraparib	[50]
ARIEL 3	To be defined	Rucaparib	

ARIEL: Assessment of Rucaparib In Ovarian CancEr TriaL; NOVA: Niraparib ovarian; SOLO: Study of olaparib in ovarian cancer.

previous courses of platinum-containing therapy and to have platinum-sensitive disease following the penultimate chemotherapy course. Patients with prior PARP therapy are excluded from participation.

Lastly, Clovis Oncology is exploring the use of rucaparib in patients with recurrent, platinum-sensitive serous ovarian cancer. Assessment of Rucaparib In Ovarian CancEr TriaL (ARIEL 2) [51] and ARIEL 3 were designed in parallel, with ARIEL 2 serving to help identify patients most likely to respond to rucaparib therapy in the Phase II setting. Ultimately, ARIEL 3 will then randomize patients 2:1 (estimated enrollment: n = 540) to rucaparib versus placebo. In an effort to better predict response and maximize efficacy, the effects of the drug will be evaluated in molecularly defined groups based on proprietary biomarker examination.

Future directions

As with all novel therapeutics, the discovery and validation of biomarkers predictive of response are crucial and recommended by the US FDA for approval of new agents [37]. In the studies reviewed above, germline *BRCA1* or *BRCA2* mutations have been validated as predictive biomarkers based on our understanding of synthetic lethality in the context of PARP inhibition. Importantly, however, patients who lack germline *BRCA* mutations but exhibit the BRCAness phenotype have also been shown to benefit from PARP inhibition, and enriching for responders is a clinical priority, as the benchmark for drug approval continues to rise.

To date, several molecular indicators of PARP responsiveness have emerged, all of which are linked to DNA repair. The histone protein H2AX concentrates at sites of DNA DSBs, while RAD51 is required for assembly of HR proteins at site of DNA damage [52,53]. Developing antibodies directed against these targets may allow for the assessment of PARP inhibitor activity during treatment. Patients who exhibit a clinical response on treatment and show increased phosphorylated H2AX or RAD51 via immunofluorescence may be triaged to continue treatment while others with low expression and mixed response are preferentially transitioned to an alternate drug.

Furthermore, understanding the mechanism of acquired resistance is essential in an effort to subvert the success serous ovarian cancer has exhibited in evading prior novel therapeutic paradigms. Currently, reversion to *BRCA* wild-type status following secondary mutations is well recognized as conferring resistance to PARP inhibition. More novel mechanisms, including the loss of function of 53bp1 – a protein involved in NHEJ – have also been identified.

The future of PARP inhibition will likely include the use of this class of drug as both a single agent and in combination with biologics and cytotoxics. Drugs resulting in DNA damage or replication fork injury as well as antiangiogenic agents have been investigated in conjunction with PARPi in the clinical setting. Both cediranib and bevacizumab result in tissue hypoxia and DNA damage. Phase I and II studies are currently exploring combining the PARP inhibitors with antiangiogenic agents [54]. Additionally, the efficacy and toxicity of PARP inhibitors in combination with the cytotoxic chemotherapeutic drugs carboplatin and paclitaxel have been examined. Dose reductions reflected likely overlapping toxicity with no survival benefit in the combined treatment phase.

Conclusion

In summary, PARP inhibitors represent a novel therapeutic class of antineoplastic agents, with significant efficacy, particularly in the *BRCA*-mutant population, and manageable toxicity. No agent is FDA approved for use in patients with serous ovarian cancer, although with four separate Phase III trials due to mature over the coming 2 years, this will likely change. In the interim, the importance of identifying biomarkers predictive of response is implicit as we look to improve patient selection, particularly within the BRCAness population and advance oncologic outcomes.

Expert commentary

The development of PARP inhibitors in the treatment of advanced stage ovarian cancer has now entered the Phase III arena. Oncologists anxiously await the results of these trials as targeted agents have emerged as effective therapeutic options. As our understanding of HR deficiency evolves, it is anticipated that a greater proportion of ovarian cancer patients will benefit from PARP inhibition.

Five-year view

In 2005, two landmark studies were published detailing the manner in which the DNA repair machinery can be targeted in *BRCA*-deficient cell lines. Since that time, five pivotal Phase II clinical trials were completed with promising response rates and manageable toxicity profiles. Currently, SOLO1, SOLO2 and niraparib ovarian are recruiting patients onto Phase III trials exploring the clinical efficacy of PARP inhibition. Additionally, ARIEL 3 will look to explore the therapeutic efficacy of rucaparib after the completion of a run-in Phase II study (ARIEL 2). We eagerly await the results of these Phase III studies, which have potential practice changing implications.

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Key issues

- Homologous recombination deficiency is estimated to occur in up to 24% of patients with advanced stage ovarian cancer.
- Poly-ADP ribose polymerase (PARP) inhibition has been identified as a novel therapeutic option in patients with homologous recombination deficiency.
- Current Phase II clinical trials show promising response rates with manageable toxicity profile.
- Three Phase III clinical trials are currently enrolling patients to study the impact of PARP inhibition on oncologic outcome.
- Moving forward, the identification of patients most likely to respond to PARP inhibition is critical, while working to identify mechanisms of acquired resistance.
- Additional research is needed to help expand our understanding of the contribution of non-*BRCA* mutations on homologous recombination and sensitivity to PARP inhibition.

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