PARP Inhibitors in Ovarian Cancer

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Poly (ADP-ribose) polymerase (PARP) inhibitors are a novel class of therapeutic agents that target tumors with deficiencies in the homologous recombination DNA repair pathway. Genomic instability characterizes high-grade serous ovarian cancer (HGSOC), with one half of all tumors displaying defects in the important DNA repair pathway of homologous recombination. Early studies have shown significant efficacy for PARP inhibitors in patients with germline breast related cancer antigens 1 and 2 (*BRCA1/2*) mutations. It has also become evident that *BRCA* wild-type patients with other defects in the homologous recombination repair pathway benefit from this treatment. Companion homologous recombination deficiency (HRD) scores are being developed to guide the selection of patients that are most likely to benefit from PARP inhibition. The choice of which PARP inhibitor is mainly based upon the number of prior therapies and the presence of a *BRCA* mutation or HRD. The identification of patients most likely to benefit from PARP inhibitor therapy in view of HRD and other biomarker assessments is still challenging. The aim of this review is to describe the current evidence for PARP inhibitors in ovarian cancer, their mechanism of action, and the outstanding issues, including the rate of long-term toxicities and the evolution of resistance.

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1. Introduction

Ovarian cancer is composed of three histological subtypes: epithelial (90%), germ cell (5%), and sex cord stromal cell (5%). Epithelial ovarian cancer (EOC) is the most lethal gynecological disease due to lack of screening test sensitivity. Histologic subtypes of EOC include high- and low-grade serous (75–80%), mucinous (3%), endometrioid (10%), and clear cell (10%). Typically, EOC is diagnosed being progressed to an advanced stage with the involvement of the peritoneal cavity and other organs. Consequently, the prognosis of the disease is dismal.

Current first-line treatment of high-grade EOC includes debulking surgery, followed by platinum-based doublet chemotherapy. Although the disease is chemo-sensitive, most patients will eventually experience disease recurrence, following the initial remission. Poly (ADP-ribose) polymerase (PARP) inhibitors have entered into standard treatment for EOC, based on the results from randomized clinical trials demonstrating the significant prolongation of progression-free survival (PFS), accompanied by acceptable tolerability. Olaparib and rucaparib have currently been approved for the treatment of recurrent *BRCA* mutant ovarian cancer, as well as maintenance

therapy in platinum-sensitive relapsed disease, whereas niraparib is only indicated for the maintenance setting. Other newer agents, such as talazoparib and veliparib, are in earlier stages of development. In the era of precision medicine, *BRCA* and homologous recombination deficiency (HRD) status represent novel predictive biomarkers of response to chemotherapy and PARP inhibitors. Germline mutations of the genes *BRCA1/2* are related to increased cancer predisposition and they account for approximately 14% of EOCs. These genes encode proteins with a crucial role in the repair of double-strand DNA breaks (DSBs) through HRD. Furthermore, somatic mutations and epigenetic inactivation of *BRCA1/2* have been implicated in sporadic ovarian cancer. Beyond *BRCA1/2*, additional genes are involved in homologous recombination DNA repair. From the clinical point of view, the efficacy of PARP inhibitors includes both germline *BRCA*-mutated ovarian cancer and sporadic ovarian cancers with HRD.

It took more than 10 years from the discovery of the synthetic lethality upon PARP inhibition to the regulatory approval of PARP inhibitors. Taken the significant improvement in patients' benefit observed in earlier therapeutic settings, along with the likelihood of long-term tolerability of PARP inhibitors, there is great potential for this drug class to become a foundation treatment for ovarian cancer, and far beyond *BRCA1/2* mutant tumors.

PARP inhibitors have changed the therapeutic strategy of patients with *BRCA*-related ovarian cancer. These agents have many similarities, but at the same time notable differences, which are based on the differences between their chemical structural features. All of the PARP inhibitors that were developed in EOC are PARP-1 and PARP-2 inhibitors, while olaparib and rucaparib additionally inhibit PARP-3. Furthermore, rucaparib inhibits tankyrase-1, which is another member of the PARP family. Currently, novel agents are in clinical development.

2. Olaparib

Olaparib is the first inhibitor of the PARP enzymes 1, 2, and 3 (PARP-1, PARP-2, and PARP-3 respectively) developed in ovarian cancer. It has been approved by both European Medicines Agency (EMA) and United States (US) Food and Drug Administration (FDA) as maintenance treatment for recurrent EOC, fallopian tube, or primary peritoneal cancer, following complete or partial response to chemotherapy with a platinum compound in patients with somatic or germline mutations in *BRCA1/2*.

Study 19 was a randomized, double-blind, placebo- controlled, phase II study of the use of olaparib as maintenance treatment in the setting of recurrent, platinum sensitive EOC. The patients were eligible, regardless of *BRCA* mutation status, and they should have been treated with two or more prior lines of platinum based therapy with complete or partial response to their most recent platinum regimen. Two hundred sixty-five patients were enrolled and randomized to olaparib 400 mg bid or placebo. Those that were treated with olaparib had a significantly prolonged PFS (8.4 months vs. 4.8 months, hazard ratio (HR) 0.35; 95% confidence interval (CI) 0.25–0.49; $p < 0.001$). This PFS advantage was prominent in all subgroup analyses. A retrospective preplanned analysis of the data by *BRCA* mutation status revealed significant improvement in PFS in patients with either germline, or somatic *BRCA* mutations that were treated with olaparib as compared with placebo (11.2 vs. 4.3 months, HR 0.18; $p < 0.0001$). However, there is a proportion of patients without a *BRCA* mutation that may also benefit from olaparib (7.4 vs. 5.5 months, HR 0.54; $p = 0.0075$). This PFS advantage was similar in a subgroup analysis of the

10% of patients with a somatic rather than germline *BRCA* mutation (HR 0.23 vs. 0.17 in somatic vs. germline *BRCA* mutations, respectively). Importantly, olaparib prolonged the time to second subsequent therapy in both *BRCA*-mutated (HR 0.44; $p < 0.001$) and non-*BRCA*-mutated patients (HR 0.64; $p < 0.34$), which suggested that treatment with PARP inhibitors did not affect further response to chemotherapy. This evidence confirmed the hypothesis that HRD and consequent susceptibility to platinum compounds or even other drugs that either create DNA damage, such as pegylated liposomal doxorubicin, or prevent damage repair, like PARP inhibitors, depend on various gene alteration beyond *BRCA* mutation. Following the results of Study 19, the EMA approved olaparib for maintenance treatment of patients with platinum-sensitive relapsed *BRCA*-mutated HGSOC who responded to platinum-based chemotherapy.

In December 2014, olaparib also received FDA-approval, for use in patients with germline *BRCA*-mutated HGSOC, fallopian tube, or primary peritoneal carcinoma following three or more prior lines of treatment. This was based on the results of Study 42, evaluated olaparib in the dosage of 400 mg bid in heavily pretreated patients with recurrent, platinum resistant ovarian cancer, and a germline *BRCA1/2* mutation, until disease progression or unacceptable toxicity. The overall response rate (ORR) in *BRCA*-mutated ovarian cancer was 34% (range 26%–42%) with a median duration of response of 7.9 months (5.6–9.6 months).

More recently, the international phase III SOLO2 study evaluated olaparib as maintenance therapy in platinum-sensitive, relapsed ovarian cancer patients with a *BRCA1/2* mutation treated with at least two lines of previous chemotherapy. Two hundred ninety-five patients were randomized to receive 2:1 olaparib tablets 300 mg orally bid or placebo after at least four cycles of platinum based regimens. Maintenance therapy with olaparib demonstrated a dramatic improvement in PFS of 19.1 months versus 5.5 months (HR 0.3; 95% CI 0.22–0.41) in treated patients using 300 mg (two tablets) bid formulation. The results of SOLO2 confirmed Study 19. In view of these data, the FDA recently granted a second therapeutic indication on August 17, 2017, for maintenance following a complete or partial response to platinum-based chemotherapy, regardless *BRCA* mutation status. Subsequently, EMA approved olaparib in maintenance setting in May 2018, based on the multicenter, randomized, double blinded phase III SOLO-1 trial. The final results were presented at the European Society for Medical Oncology 2018 Congress. Four hundred fifty-one *BRCA1/2* mutated patients were randomized in a 2:1 ratio to first-line maintenance with olaparib 300 mg bid or placebo. The median PFS for patients on the placebo arm was only 13.8 months, while the median PFS for those that were treated with olaparib was not reached, but looks to be approximately three years longer than the placebo group (HR 0.30; $p < 0.0001$).

3. Niraparib

Niraparib is a potent and selective inhibitor of PARP-1 and PARP-2. It is primarily metabolized by carboxylesterase to form a major inactive metabolite, which subsequently undergoes glucuronidation. The activity and safety of niraparib monotherapy 300 mg OD were initially assessed in the phase I trial, including 42 patients with relapsed ovarian cancer. Pharmacodynamic analyses confirmed that PARP inhibition exceeded 50% at daily doses that were greater than 80 mg and antitumour activity was confirmed beyond doses of 60 mg. The ORR in *BRCA1/2* mutated patients was 40%, and the relevant median duration of response 12.9 months. Interestingly, an ORR of

67% was achieved in platinum-sensitive disease and *BRCA1/2* wild-type patients. These results are compatible with those that were observed in a double-blind, randomized phase III study, investigating the role of niraparib as maintenance therapy in relapsed ovarian cancer. In this trial, 553 EOC patients were randomized 2:1 to orally niraparib 300 mg QD or matched placebo within eight weeks of the most recent therapy, until progression or unacceptable toxicity. All of the patients had been treated with at least two prior platinum-based regimens. The eligible patients were assigned to one of two cohorts; those with germline *BRCA* mutations were assigned to the germline *BRCA* mutated cohort (n = 203), whereas patients without germline *BRCA* mutations were assigned to the non-germline *BRCA* mutated cohort (n = 350). In the primary efficacy analyses, the *BRCA* wild-type cohort was divided into two subgroups according to HRD status, which were based on companion diagnostic test for HRD. Furthermore, in exploratory analyses, the HRD-positive subgroup was further defined by the presence or a lack of a somatic *BRCA* mutation, respectively (47 patients HRD-positive somatic *BRCA1/2* mutated and 115 patients HRD-positive *BRCA* wild-type). The PFS was independently assessed in the germline *BRCA* and *BRCA* wild-type cohort, and it was found to be longer among the niraparib-treated patients in all groups when compared to the placebo. Among germline *BRCA* mutation carriers, there was a significant prolongation in PFS in the niraparib group as compared to placebo (median PFS 21.0 months vs. 5.5 months, HR 0.27; 95% CI 0.017–0.41; p < 0.0001). When the HRD tumors were retrospectively assessed in an exploratory analysis out of the non-germline *BRCA* group, niraparib reduced the risk of progression by 62% (PFS 12.9 months versus 3.8 months; HR 0.38; 95% CI 0.24–0.59). Non-germline *BRCA* mutant and negative HRD patients that were treated with niraparib achieved a smaller, but significant, prolongation of PFS when compared to placebo (9.3 months vs. 3.9 months, HR 0.45; 95% CI 0.34–0.61). Furthermore, PFS was longer in the HRD-positive somatic *BRCA1/2* mutated subgroup, similarly to the germline *BRCA* cohort (20.9 vs. 11.0 months, HR 0.27; p = 0.02). In the HRD-positive *BRCA* wild-type subgroup, PFS was 9.3 and 3.7 months in the niraparib group and in the placebo group (HR 0.38; p < 0.001), respectively. Finally, in the HRD-negative non-germline *BRCA* mutation subset of patients, the obtained PFS was 6.9 vs. 3.8 months (HR, 0.58; 95% CI 0.36–0.92; p = 0.02).

In March 2017, FDA approved niraparib at a dose of 300 mg daily as maintenance treatment of recurrent EOC, fallopian tube, or primary peritoneal cancer, in responders to platinum-based chemotherapy; the approval of niraparib by EMA, at the same context, came in November 2017. Furthermore, in June 2018, the results of the Quadra Trial were presented at the American Society of Clinical Oncology annual meeting. This was a phase II, open-label study of niraparib in the setting of platinum sensitive, HRD-positive, HGSOC. Among the 45 patients treated with three or more previous regimens without prior PARP inhibitor administration, the achieved ORR was 27.5% and the duration of response 9.2 months. Finally, PRIMA (NCT02655016) is an ongoing, phase III, randomized, placebo-controlled study of maintenance niraparib in high-risk patients with HRD advanced ovarian cancer, who responded to first-line platinum-based chemotherapy.

4. Rucaparib

Rucaparib is a potent PARP-1 and PARP-2 oral inhibitor, which is approved by FDA in December 2016 and by EMA in May 2018 as monotherapy for the treatment of advanced *BRCA*-mutated ovarian cancer, relapsed after at

least two chemotherapy lines. It differs by other PARP inhibitors, because it inhibits tankyrases that promote homologous recombination. Rucaparib is metabolized by *CYP2D6* and, to a lesser extent, by *CYP1A2* and *CYP3A4*. It has been evaluated in two key clinical trials in this setting; the phase I II Study 10 and the phase II trial ARIEL 2.

Part 1 of Study 10 (phase I) included 56 patients with advanced solid tumors and established an optimal dose of 600 mg bid, which is characterized by acceptable toxicities. In Part 2A (phase II), 42 patients with recurrent, platinum-sensitive ovarian cancer, and germline *BRCA1/2* mutations, who were previously treated with two to four lines of chemotherapy, received maintenance rucaparib 600 mg bid. The reported objective response rate was 59.5% and the median duration of response of 7.8 months (range 5.6–10.5).

The ARIEL 2 study was a multicenter, two-part, phase II open label trial that assessed the efficacy of rucaparib in relapsed HGSOC or endometrioid ovarian carcinoma following one or more (part 1) and three or four prior chemotherapy lines (part 2), independently of the platinum-sensitivity status. ARIEL 2 Part 1 enrolled 192 platinum-sensitive OC patients and stratified the patients into three cohorts; *BRCA1/2*-mutated (n = 40), *BRCA* wild-type with loss of heterozygosity (LOH) high (n = 82), and *BRCA* wild-type with LOH low (n = 70). The median PFS was significantly longer in the *BRCA* mutated subgroup (12.8 months) and in the *BRCA* wild-type LOH high (5.7 months) when compared to *BRCA* wild-type LOH low subgroup (5.2 months). This difference was significant in the *BRCA* mutant group (HR 0.27; 95% CI 0.16–0.44; $p < 0.0001$) when compared to the LOH low group; a similar, though not statistically significant, trend was demonstrated in the LOH high group (HR 0.62; 95% CI 0.42–0.90; $p = 0.011$) as compared to the LOH low group. There was also a notable advantage in the median duration of response in the *BRCA* mutant group (9.2 months) and LOH high group (10.8 months) as compared to the LOH low group (5.6 months), whereas, the ORR was higher in the *BRCA1/2*-mutated (80%) and in the *BRCA* wild-type LOH high (29%) than the *BRCA* wild-type LOH low subgroup (10%). Indeed, this study is the first that prospectively demonstrated that the HRD signature could serve as a predictive biomarker for the PARP inhibitor response in *BRCA* wild-type patients with HGSOC. Interestingly, an analysis of tumor biopsies revealed an association between the methylation of *BRCA1* or *RAD51C* and high LOH, with positive impact on ORR (52.4% and 75%, respectively) and PFS (7.4 months and 11.1 months, respectively). However, the establishment of the predictive value of methylation of *BRCA1* or *RAD51C* requires further study. The second part of ARIEL 2 trial is still ongoing (NCT01891344); 286 patients who have been treated with at least three instances of chemotherapy and recurred with both platinum sensitive or resistant disease have been enrolled with the prospect to of evaluating the clinical activity of rucaparib based on HRD status. Preliminary data incorporating Parts 1 and 2 demonstrate a difference in PFS among *BRCA* mutation carriers on the basis of platinum sensitivity (12.7, 7.3, and 5.0 months in platinum-sensitive, –resistant, and –refractory setting, respectively). The pooled analysis of the two trials confirmed these encouraging results, including 106 patients with HGSOC and a deleterious *BRCA1/2* mutations. Among them, 42 patients participated in Study 10 (Part 2A) and 64 in both parts of ARIEL 2. All of the patients were treated with at least two prior lines of chemotherapy, while 74.5% exhibited sensitivity to their last platinum-based therapy, 18.8% were platinum resistant, and 8.4% platinum refractory. Germline mutations were detected in 83% all patients, whereas 17% were carriers of somatic alterations. Among them, *BRCA1/2* genes were identified in 63.2 and 36.8%, respectively. The median duration of response was 9.2 months, and no difference in ORR was reported

between the *BRCA1/2* mutated subgroups. Additionally, patients with a platinum-free interval that exceeded 12 months had a higher ORR than those with a platinum-free interval of 6–12 months or less than six months.

ARIEL 3 is a phase III randomized trial of oral rucaparib 600 mg bid versus placebo (2:1 randomization) as maintenance treatment in 564 patients with platinum-sensitive HGSOC or endometrioid ovarian cancer, in response to their recent platinum-based chemotherapy. The HRD signature that was mentioned in ARIEL 2 is also being prospectively assessed in ARIEL 3. The primary endpoint was the PFS, which was evaluated based on the molecular signatures of *BRCA* mutations (germline or somatic), HRD-positivity (including *BRCA* mutant and *BRCA* wild-type with LOH high), and intent-to-treat (all enrolled patients). As such, for BRCA mutated patients, the median PFS was 16.6 months in the rucaparib arm versus 5.4 months in the placebo group (HR 0.23; $p = 0.0001$). Similarly, in BRCA wild-type patients, the reported PFS for those with an HRD-positive disease treated with rucaparib was 13.6 months as compared to 5.4 for the placebo group (HR 0.32; $p = 0.0001$), while, in the intention to treat population, it was 10.8 versus 5.4 months, respectively (HR 0.36; $p = 0.0001$). The secondary endpoint of blinded independent central review PFS was also significant for each molecular signature separately: germline *BRCA* mutation (26.8 months vs. 5.4 months), homologous recombination deficient high LOH (22.9 months versus 5.5 months), and overall intent-to-treat populations (13.7 months vs. 5.4 months). An exploratory analysis in the *BRCA* wild-type only revealed a maintained benefit of rucaparib in both the HRD-positive (median PFS 9.7 months, HR 0.44; $p < 0.0001$) and HRD-negative subsets (median PFS 6.7 months, HR 0.58; $p = 0.0049$).

Finally, ARIEL 4 (NCT02855944) is an ongoing phase III study, which was designed to compare the efficacy and the safety of rucaparib versus physician's choice of chemotherapy, depending on platinum status, in *BRCA1/2* mutated recurrent ovarian cancer following at least two previous lines of systemic treatment. Primary endpoint is PFS with a target enrollment of 345 patients. Rucaparib is also being explored in combination with programmed death-ligand 1 (PD-L1) inhibitor atezolizumab in a phase Ib trial of patients with recurrent, platinum-sensitive ovarian cancer (NCT03101280).

5. Veliparib

Veliparib is a potent inhibitor of PARP1/2, which was demonstrated in 2007 to have high anti-tumor efficacy when combined with DNA alkylating agents, such as temozolomide and irradiation. Even though evidence supporting the use of veliparib in the treatment of EOC is limited as compared to other PARP inhibitors, there are still several ongoing studies, with veliparib, either as monotherapy, or, combined with chemotherapy.

In GOG 280, a phase II, single-arm trial, veliparib 400 mg bid was administered to 50 *BRCA* mutated patients with persistent or recurrent ovarian cancer, which were previously treated with up to three prior lines of chemotherapy, until progression or intolerance The ORR to veliparib was 26% (90% CI: 16–38%); nevertheless, 31 out of 50 patients (61%) progressed on treatment, whereas response in the platinum-resistant and platinum-sensitive patients was 20 and 35%, respectively, with a median PFS of 8.2 months (ranging from 0.43 to 19.55 months) and a median OS of 19.7 months (ranging from 2.3 to 19.7 months). A phase I trial evaluated the maximum tolerated dose, pharmacokinetic and pharmacodynamics properties, and clinical response of veliparib. The recommended

phase 2 dose was 400 mg bid, whereas the ORR in *BRCA* mutated, and *BRCA* wild-type patients was 23% and 4%, respectively, accompanied by a clinical benefit rate of 58% and 38%, across all dose levels. A more recent phase I II trial assessed the role of single agent veliparib in patients with germline *BRCA* mutations, in the setting of platinum sensitive or resistant ovarian cancer. Sixteen participants were enrolled in the phase I study and 32 in the phase II. The maximum tolerated dose was established at 300 mg twice daily. Median PFS and overall survival (OS) for the intention to treat population were 5.6 and 13.7 months, respectively.

A phase II trial randomized 72 patients with *BRCA*-mutated ovarian cancer to oral cyclophosphamide 50 mg daily with or without veliparib 60 mg PO daily. The combination of veliparib with cyclophosphamide did not improve ORR (11.8% vs. 19.4%, respectively) or median PFS (2.1 months vs. 2.3 months, respectively; $p = 0.68$) as compared to single agent cyclophosphamide. Similarly, no responses were reported in phase I study of veliparib in combination with topotecan. GOG 3005, a double-blind, randomized phase III trial for the evaluation of veliparib as first-line treatment in association with carboplatin and paclitaxel in newly diagnosed patients with stage III IV EOC is currently recruiting participants ($n = 1140$; NCT02470585). The study includes three treatment arms: chemotherapy only, chemotherapy followed by veliparib switch maintenance, and veliparib combined with chemotherapy followed by continuation maintenance.

In a phase I trial, Reiss et al. evaluated the activity of veliparib combined with low-dose fractionated whole abdominal radiation therapy in 32 patients with peritoneal carcinomatosis due to advanced solid tumors. Among them, 18 were patients with EOC, five of whom with *BRCA* mutations. The reported ORR was only a 3%, while a stable disease was exhibited by 33% of patients. Those with *BRCA* mutated EOC had a PFS of 4.47 months, as compared to 3.58 months for the *BRCA* wild-type cohort and an OS of 10.15 months versus 7.89 months, respectively. The PFS of the patients with platinum-sensitive disease was 7.92 months, while those with platinum-resistant EOC reached 3.58 months.

6. Talazoparib

Talazoparib is a PARP1/2 inhibitor, which is selective against *BRCA1/2* and phosphatase and tensin homolog (*PTEN*) mutants, enhances the cytotoxic activity of temozolomide, SN-38, and carboplatin. The efficacy of talazoparib in the treatment of ovarian cancer is still under investigation. It initially assessed in a two-stage dose-escalation trial study, with the enrolment of 34 EOC patients, of whom 25 with germline *BRCA* mutations, all being previously treated with platinum-based chemotherapy. The reported ORR was 42% and median PFS 36.4 weeks in a well-tolerated dose of 1000 μg day. More recently, a phase I study evaluated talazoparib in combination with carboplatin in patients with several solid tumors independently of germline status. Among the 24 participants, two had EOC (8%), while 20% identified *BRCA1/2* mutations. In the subset of *BRCA* mutated patients, 1 and 2 cases achieved complete and partial responses, respectively, whereas three patients with somatic *BRCA* mutation maintained a stable disease beyond four months.

The benefit of talazoparib has been established in a phase II ABRAZO study in terms of patients specifically with *BRCA* mutations. Enrolment was restricted to patients with breast cancer and the reported ORR for *BRCA1* and

BRCA2 mutations carriers was 24% and 34%, respectively. However, data from phase II or III studies in patients with EOC are still not available. Nevertheless, a phase II trial of talazoparib in recurrent *BRCA1/2*-mutated ovarian cancer patients, following primary progression on prior PARP inhibitor, has recently been completed (NCT02326844). It will be interesting to be clarified whether there is a role for further PARP inhibition in this context.

Currently, there are several trials actively recruiting patients evaluating talazoparib either as monotherapy or in combination. With this regard, a phase 1 study assesses the combination with a checkpoint inhibitor (NCT03330405), whereas, a phase II study exploring talazoparib activity in advanced cancers with PTEN mutations or *PTEN* loss and HRD defect (NCT02286687). Furthermore, an ongoing phase II randomized study (NCT02836028) evaluates the activity and tolerance of single agent talazoparib versus combination with temozolomide in patients with *BRCA*-mutated or homologous recombination-deficient relapsed ovarian cancer.

7. Functional Aspects of PARP1

PARP1 initiates and modulates multiple DNA repair pathways, and it is thus important for maintaining genomic integrity. Transcriptional regulation by PARP1 involves both ADP-ribosylation-dependent and independent mechanisms. PARP1 might also regulate transcription by modulating the chromatin structure, altering DNA methylation patterns, acting as a co-regulator of transcription factors, and interacting with chromatin insulators. Under physiological conditions, PARP1 ADP-ribosylation activity curiously follows the rhythmic circadian cycle.

The interaction between PARP1 and the NF-κB pathway promotes the production of several pro-inflammatory cytokines, including TNFα, IL-6, INFγ, E-selectin, and ICAM-1; PARP inhibition attenuates the upregulation of these factors in response to inflammatory stimuli, and in parallel prevents inflammation-associated side effects of cytotoxics. The loss of PARP1 activity inhibits proliferative signaling and metastasis through anti-inflammatory mechanisms.

PARP1 also regulates the c-Jun N-terminal kinase (JNK) pathway, which is implicated as a driver of both tumor development and treatment response. PARP1 downregulates MAP kinase phosphatase MKP-1 expression and inhibits the survival kinase Akt, both of which activate JNK. Based on that, PARP inhibition could be potentially therapeutically beneficial in ovarian cancer taken the elevated JNK activity. PARP1 inhibitors promote Akt activity and mTOR signaling, which leads to decreased cell death.

In addition to the JNK-mediated signaling, extracellular signal-regulated kinases (ERKs) represent a second family of MAP kinases that participate in cell death determination, tumor progression, angiogenesis, and metastasis. ERK activation is pivotal in cancer cell survival through the upregulation of anti-apoptotic proteins and inhibition of caspase activity. The jnhibition of this pathway by targeting ERK or MEK leads to suppression of ovarian tumor growth. Indeed, PARP1 inhibition causes a loss of ERK2 stimulation by decreasing the activity of critical pro-angiogenic factors, including vascular endothelial growth factor (*VEGF*) and hypoxia inducible factor (*HIF*).

8. Resistance

Advances in the understanding of PARP inhibitors' resistance is of paramount importance, and it may lead to novel insights into basic mechanisms of the DNA damage response. Each PARP inhibitor has separate chemical structure with diverse off-target effects. This indicates that the utilization of a secondary PARP inhibitor could be therapeutically beneficial in a resistant tumor. The restoration of homology-directed DNA repair through secondary reversion mutations is the most common identified mechanism of resistance. Indeed, the restoration of *BRCA* activity starts from *BRCA*-deficient and chemo-sensitive cells as a result of several mutations that are induced by platinum agents. This initial restored clone expands in the setting of treatment-specific selective pressure. In this context, somatic *BRCA1/2* mutations were predicted to restore the protein function in the germline *BRCA1/2* mutated ovarian cancer patients in a study. Among 46 women that were exposed to tumor sequencing, 28% (13 out of 46; 95% CI 17.3–42.6%) possessed secondary *BRCA* mutations that were predicted to restore *BRCA* function and homologous recombination activity.

Compensatory deleterious mutations have also been detected to confer PARP inhibitor resistance. In contrast to the homologous recombination, NHEJ only involves minor resection of DNA ends at regions of DSB. *TP53* binding protein 1 (*53BP1*) maintains the balance between homologous recombination and NHEJ, and it promotes NHEJ through the inhibition of extensive DNA end-resection that is required for homologous recombination repair. As such, the loss of *53BP1* function by either mutation or downregulation accelerates the *BRCA1*-independent end-resection and provides PARP inhibitor resistance. It has been demonstrated that the inactivation of downstream factors of *53BP1*-mediated repair, typically *RIF1* and *REV7*, also leads to the restoration of DNA end resection, and consequently promotes homology-mediated repair. In vitro studies revealed that the loss of *53BP1* function allows for the partial restoration of homologous recombination in *BRCA1*-deficient cells and counteracts sensitivity to the PARP inhibitor. Heat shock protein 90 (*Hsp90*) is a crucial molecular chaperone that functions to correctly fold client proteins, and consequently prevents them from degradation by the ubiquitin-proteasome system. In vivo synergism between an *HSP90*-inhibitor (AT13387) and olaparib in PARP inhibitor resistant ovarian cancer has been described. Alternatively, the evidence that acquired epigenetic changes, such as hypermethylation promoter of *BRCA1*, may restore normal *BRCA1* protein expression levels.

Furthermore, epigenetic silencing or accelerated protein synthesis and degradation could also lead to decreased expression of PARP enzymes, followed by PARP inhibitors resistance. Another mechanism of inherent or acquired resistance is the upregulation of genes encoding p-glycoprotein efflux pumps, related to decrease intracellular drug levels. In a murine breast cancer model, olaparib resulted in initial inhibited tumor growth, which is associated with an impressive increase in the expression of p-glycoprotein efflux pumps. This resistance can be reverted by the *ABCB1* inhibitors verapamil, elacridar, and tariquidar. However, toxicity and lack of specificity characterize *p-glycoprotein* inhibitors.

Additional pharmacologic methods for reversing PARP inhibitor resistance have been investigated. The knockdown of cyclin-dependent kinase 12 (*CDK12*) resulted in concomitant downregulation of DNA repair proteins, and consequently the development of a "*BRCA*ness" phenotype. There is in vitro evidence that pharmacological

inhibition of *CDK12* with Dinaciclib reverses acquired PARP inhibitor resistance. Furthermore, it has been shown that the inhibition of cell cycle regulator *WEE1* leads cells to enter the S-phase of the cell cycle, and therefore to further the accumulation of DNA DSBs in the context of HRD and PARP inhibition. Overall, a combined inhibition of *CDK12* or *WEE1* could be a strategy that is recommended for overcoming homologous recombination restored PARP inhibitor resistance.

9. Future Perspectives

PARP inhibitors are a new class of biologic agents, which have changed the clinical management of the ovarian cancer, based upon the pre-selection characteristics of the tumors. It has been established that they have improved PFS, although a longer follow-up is required to also assess the prolongation of OS. Numerous clinical trials are ongoing, both for the currently available PARP inhibitors and those that have not yet been approved by the FDA. The analysis of *BRCA* mutational status represents a step forward to the individualized management of patients with ovarian cancer, and it should be incorporated in their diagnostic approach. Defects in homologous recombination repair seem to confer PARP inhibitors sensitivity. However, the understanding of mechanisms that contribute to clinical PARP inhibitor responses in the absence of HRD is still under investigation. The increased availability of PARP inhibitor treated specimens will potentially provide insight into novel biomarkers and acquired resistance mechanisms. It appears that treatment with PARP inhibitors is effective for patients with either germline, or somatic *BRCA1/2* mutations. The future challenge will be the optimal choice of PARP inhibitor at any given time. That demands the design of larger phase III trials, with head-to-head comparisons of them. Furthermore, PARP inhibitors have unique AEs that require further evaluation. Usually, toxicity is easily managed with supportive care and dose reduction, or modification. Further research is needed in terms of the combination of PARP inhibitor, with antiangiogenic, immunocheckpoint inhibitors, and cytotoxics as strategies for overcoming resistance mechanisms, potentiating the therapeutic efficacy, and expanding their clinical utility in non-homologous deficient tumors.